Eur Biophys J (2007) 36 (Suppl 1):S51–S248
DOI 10.1007/s00249-007-0178-7

Abstracts

– Plenary Lectures –

O-1

Oxygen, life and energy conversion

J. E. Walker
Medical Research Council Dunn Human Nutrition Unit, Cambridge, U.K.

Oxygen evolving life may have begun on earth about 3,400 billion years ago, but the interpretation of geochemical evidence, for example in the Buck Reef Chert, is disputed. However, evidence from fossilised stromatolites in Australia is perhaps more convincing. Modern oxygen evolving photosynthesis requires more than 200 genes. Many of the proteins are organised in highly complex structures in the chloroplasts of green plants where incident light energy from the sun is entrapped in the carbohydrates and fats that provide our food with calorific value. We release the energy by respiration (controlled burning), consuming in the process most of the oxygen that we have breathed in. More than 1000 proteins are involved in cellular respiration. About 100 or so of these proteins are organised into the respiratory enzyme complexes that function as molecule machines to convert the redox energy derived from energy in food-stuffs into adenosine triphosphate (ATP), the energy currency of biology. The final synthetic step is achieved by a remarkable molecular machine that has a mechanical rotary action. Its closest man-made analogue is the Wankel rotary engine. Its workings will be described and how such complex machines evolve will be discussed.

O-2

Single-molecule physiology of protein machines

K. Kinosita, Jr.
Faculty of Science and Engineering, Waseda University, Tokyo 169-8555, Japan

We try to understand how protein machines work by closely watching their conformational changes under an optical microscope. We attach to a selected portion of the protein machine a tag that is huge compared to the protein molecule. A huge tag allows seeing-is-understanding type experiments, and also serves as a handle for manipulation. To visualize how a two-legged molecular motor myosin V walks, we attached a microtubule a few μm in length to one of the legs. We found that the leg, when lifted from the actin track, undergoes extensive Brownian rotation in all directions, indicating the presence of a free joint at the leg-leg junction. After landing, the leg leans forward to bias the random rotation of the other leg forward. To elucidate the chemo-mechanical coupling in the rotary motor F1-ATPase, we attached a 40-nm gold particle to the rotor subunit to resolve its rotation at 8,000 video frames/s. This motor proceeds basically in steps of 120° per ATP hydrolyzed, and now we show that the last 40° portion is driven by phosphate release and that phosphate can reverse the 40° substep. Previously we reported that the first 80° rotation is driven by ATP binding, but imaging of binding/release of a fluorescent ATP analog while the rotor is slowly rotated by magnets suggests that ADP release, nearly simultaneous with ATP binding, also drives the 80° substep. These findings together with previous results complete the basic coupling scheme of this motor.

O-3

Structure and mechanism of membrane transporters

S. Iwata
Division of Molecular Biosciences, Imerial College London, UK; Diamond Light Source, UK; ERATO Iwata Project, Japan; Kyoto University, Japan

Membrane transport proteins that transduce free energy stored in electrochemical ion gradients into a concentration gradient are a major class of membrane proteins. We have reported the crystal structure at 3.5 Å of the Escherichia coli lactose permease (LacY), an intensively studied member of the Major Facilitator Superfamily of transporters. The structure with a bound lactose homologue, β-D-galactopyranosyl-1-thio-β-D-galactopyranoside (TDG), reveals the sugar-binding site in the cavity, and residues that play major roles in substrate recognition and proton translocation are identified. Recently, we determined two novel ligand-free X-ray structures of LacY at acidic (3.3 Å) and neutral pH (2.95 Å) in a different crystal forms. Based on these structures and the TDG complex structure, we propose a model for the mechanism of coupling between lactose and H^+ translocation. No sugar-binding site is observed in the absence of ligand, and deprotonation of the key residue Glu269 seems associated with ligand binding. Thus, substrate induces formation of the sugar-binding site, as well as the initial step in H^+ transduction. A possible mechanism for lactose/proton symport of LacY will be discussed. I will also update the structure studies of other membrane transpoter proteins and propose more general trasnsport mechanism for the MFS transporters.

O-4

Insight into structure and dynamics from weak alignment NMR

A. Grishaev, J. Chill, J. Ying, L. Yao, B. Voegeli, A. Bax
Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, MD 20892, USA

Spectral simplicity of solution NMR spectra results from the Brownian rotational diffusion of solutes, which rapidly averages the strong dipolar interactions between different spins to exactly zero. Much valuable structural information, contained in these dipolar interactions, is lost in this averaging process. It has long been known that alignment of solutes in a magnetically oriented liquid crystalline medium restores the dipolar interactions, albeit at the cost of dramatically increased spectral complexity, limiting this approach to only very simple systems. However, by decreasing the degree of solute alignment, it is possible to retain the valuable structural information contained in the dipolar couplings, without considerably increasing spectral complexity.

With the rapidly increasing number of previously solved macromolecular structures, the alignment approach can take advantage of this structural database by revealing which fragments are compatible with experimental dipolar couplings. This approach can provide considerable shortcuts in macromolecular structural studies, while providing a very sensitive measure to identify subtle structural changes.

Measurement of residual dipolar couplings can also provide information on the degree of order for a given interaction relative to the rest of the protein. Under favorable conditions, an absolute degree of order for various elements in the structure can be obtained, as well as information on the direction in which motions occur.

Abstracts

– Plenary Lectures –

O-5

Building cell movements step by step

C. Sykes
Laboratoire Physico-chimie "Curie", UMR168, Institut Curie – CNRS, 11, rue Pierre et Marie Curie, 75231 Paris, France

Cells move and divide by dynamically assembling and disassembling their cytoskeleton. These processes are regulated by numerous proteins from the cytoplasm, which make the mechanism very complex to understand. However, general trends can be isolated from in vivo observations. In order to unveil generic mechanisms of cell movements, we developed simplified stripped-down systems that reconstitute cellular behaviours. These systems allow for a controlled study of the physics and the biochemistry of certain types of cell movements. In particular, actin assembly is able to produce forces and deformations, with or without myosin motors. We will describe experiments illustrating how the actin-generated forces can be measured in a cell-like system, and how these forces can be modulated by physical effects triggered by actin filaments crosslinkers, or other actin-binding proteins. Moreover, we will show that actin polymerization in cells not only takes place at the plasma membrane, but might actually play an important role in the separation of vesicles from intracellular membranes.

O-6

Regulation of the cell´s dynamic city plan and the myosin family of molecular motors

J. Spudich, A. Dunn, S. Churchman, Z. Bryant, D. Altman
Dept. of Biochemistry, Stanford University, Stanford, CA 94305, U.S.A.

Molecular motors carry cargo to specific locations. Tension sensing is one fundamental regulatory element. The molecular basis of how myosin motors work has been significantly advanced by studies of myosins V and VI. Myosin V moves processively by stepping arm-over-arm, walking along the 36-nm pseudo-repeat of an actin filament by swinging its long lever arms through an angle of $\sim 70^o$, and hydrolyzing one ATP per step. *Intramolecular* tension sensing establishes a bias in the behavior of the two heads with regard to nucleotide kinetics that allows the rear head to most often release from the actin by binding ATP. We track single gold nanoparticle-myosin V conjugates using darkfield imaging with submillisecond time resolution, and directly observe the behavior of the unbound head as the motor translocates. We have also developed a technique called single-molecule high resolution co-localization (SHREC), which allows simultaneous co-localization of two chromatically differing fluorophores only 10 nm apart. We used SHREC to directly observe myosin V molecules walking hand-over-hand. We are now adapting SHREC to observe myosin V's nucleotide dynamics using dye-labeled ATP molecules. Myosin VI has a very short lever arm that is only two light chains long. Nevertheless, the molecule surprisingly steps processively 36 nm along an actin filament. Furthermore, myosin VI moves in the opposite direction to that of myosin II and myosin V. Our most recent work shows how this unusual motor achieves these feats. Myosin VI is now a paradigm of how *intermolecular* tension sensing is translated into biologically important trafficking regulation.

Abstracts

– EBSA Prize Lecture –

O-7

Studying structure and dynamics of protein complexes by solid-state NMR Spectroscopy

C. Ader, G. Angerstein, M. Etzkorn, I. de Gortari, H. Heise, R. Schneider, K. Seidel, M. Baldus
Solid-state NMR group, Max Planck Institute for Biophysical Chemistry

Solid-state NMR (ssNMR) offers structural insight into the formation of molecular complexes for a wide range of molecular sizes and binding affinities. Recent instrumental and methodological progress has enabled novel possibilities for using multi-dimensional ssNMR to study molecular 3D structures and interactions in noncrystalline systems.

In our group, such methods are used to study protein folding and aggregation on the atomic level and in a time-resolved manner for proteins involved in Alzheimer's and Parkison's disease. In addition, we have developed a set of ssNMR experiments to study molecular structure, topology and complex formation in lipid bilayers. Such techniques can be used to characterize ligand binding to G-protein coupled receptors or ion channels. In the latter case, we have shown that high affinity toxin-binding to a chimeric Kv1.3 channel involves structural rearrangements of both constituents.

Refinement of the ssNMR-derived structure of the toxin/ion channel system and recent work in larger, membrane-embedded protein complexes suggests that conformational plasticity is a central parameter defining ligand recognition and molecular complexation events in membranes.

Abstracts

– Single molecule mechanics –

P-8

Which structural elements make kinesin processive?

S. Adio[1], J. Jaud[2], B. Ebbing[2], M. Rief[2], G. Woehlke[2]
[1]National Institute for Medical Research, Physical Biochemistry, The Ridgeway, NW7 1AA London, U.K., [2]Technical University of Munich, Physics Department E22, James-Franck-Str., 85748 Garching, Germany

Kinesin-1 motor proteins travel processively along microtubules by taking hundreds of successive 'hand-over-hand' steps. They are composed of two identical subunits, each consisting of a motor domain and a neck/stalk. Structural elements responsible for the accurate coordination between the two subunits remain unknown. To identify these elements, we have constructed a chimeric motor with the motor domain of Kinesin-1 and a neck/stalk of a non-processive Kinesin-3.

This novel kinesin behaves qualitatively as conventional Kinesin-1. It moves processively in single molecule fluorescence assays and exerts up to 3 pN force in optical trapping experiments. However, reduced velocities and run lengths at a variety of loads indicate that the unconventional neck domain hinders the diffusive search for the next binding site. In the reverse chimera (motor domain of non-processive Kinesin-3 and neck/stalk of Kinesin-1) the Kinesin-1 neck allows sequential ADP release from the partner heads upon microtubule binding. But this motor was unable to make successive steps.

Our data suggest that kinesin processivity requires two independent elements. One, located in the neck/stalk region, ensures coordinated ADP release from the motor domains. The other, located in the motor domain, allows successive 'hand-over-hand' stepping by coordinating the timing of events between the two motor domains.

P-9

Nanomanipulation of single chromatin fiber with magnetic tweezers

A. Bancaud[1], G. Wagner[1], N. Conde e Silva[2], P. Recouvreux[1], C. Lavelle[4], J. Mozziconacci[3], H. Wong[3], M. Barbi[3], E. Le Cam[4], J.-M. Victor[3], J.-L. Viovy[1], A. Prunell[2]
[1]Insitut Curie CNRS-UMR 168, Paris, France, [2]Institut Jacques Monod CNRS-UMR 7592, Paris, France, [3]LPTMC CNRS-UMR 7600, Paris, France, [4]Institut Gustave Roussy CNRS-UMR 8126, Paris, France

The nucleosome core particle is the basic subunit of chromatin structure. It consists of ∼146 DNA bp coiled leftward around an octamer that contains pairs of histones H2A, H2B, H3 and H4. Magnetic tweezers were used to study the mechanical response under torsion of single nucleosome arrays reconstituted on tandem repeats of 5S or 601 positioning sequences. These fibers show higher torsional plasticity than naked DNA. This behavior can be explained by a dynamic equilibrium between three conformations of the nucleosome, corresponding to different crossing statuses of the entry/exit DNAs : negative, null or positive. Moreover these chromatin fibers, after extensive positive supercoiling, display an hysteretic behavior in their mechanical response to torsion. The fibers remain more extended when they are returning to negative supercoiling values. This hysteresis is the consequence of the trapping of one positive turn per nucleosome. The results suggest a rearrangement of the nucleosome structure which can be related with the previously documented chiral transition of the tetrasome (the H3-H4 tetramer with its bound DNA). As the energy of the altered form is ∼6 kT, these abilities to endorse torsion may be related to physiological processes such as trancription elongation since RNA polymerases generate positive supercoiling downstream.

O-10

Investigation of torque-generation in the bacterial flagellar motor

R. M. Berry, C.-J. Lo, M. C. Leake, T. Pilizota, Y. Sowa
Clarendon Laboratory, Department of Physics, University of Oxford, Parks Road, Oxford OX1 3PU, United Kingdom

1

Many bacterial species swim using flagella. The flagellar motor couples ion flow across the cytoplasmic membrane to rotation. Ion flow is driven by both a membrane potential (V_m) and a transmembrane concentration gradient. We are investigating the mechanism of the motor using a chimeric sodium-driven motor in *Escherichia coli* that allows control of the ion-motive force in combination with high-resolution measurements of motor rotation. We have shown that motors containing a single torque-generating unit take 26 steps per revolution when driven by a low sodium-motive force (smf). To characterize the stepping motion further we have developed techniques to control and measure both components of the smf in single cells, using pH and [Na^+] as control paramters and fluorescent indicators as sensors. We found V_m = 140 ± 14 mV in *Escherichia coli* at external pH 7.0, decreasing to 85 ± 10 mV at pH 5.0, and that smf could be controlled in the range 187 ± 15 mV to 53 ± 15 mV by varying pH and [Na^+] in the ranges 7.0–5.0 and 1–85 mM respectively. Rotation rates for 0.35 μm and 1 μm diameter beads attached to Na^+-driven chimeric flagellar motors varied linearly with V_m. For the larger beads the two components of the smf were equivalent, but for a given smf the speed of smaller beads increased with sodium gradient and external sodium concentration. With a smf of ∼-50 mV, motors rotate the smaller beads stably at speeds of ∼10 Hz per unit, allowing well controlled measurements of stepping.

P-11

Mechanics of the maltose-binding protein

M. Bertz, M. Rief
Physikdepartment E22, Technische Universität München, Germany

We employ single molecule force spectroscopy to investigate the mechanical behaviour of the maltose-binding protein (MBP), a large, two-domain protein serving as a periplasmic sugar sensor in *E. coli*.

In contrast to many more simple proteins previously studied by force spectroscopy, MBP populates several subsequent intermediate states on its unfolding pathway. To further elucidate this complex unfolding pattern, a cysteine cross-linking strategy was used to unambiguously map the length information obtained from unfolding experiments onto the structure of the protein. Initial unfolding proceeds from the C-terminus in a low-force, close-to-equilibrium transition resulting in an intermediate comprising the first 295 aa of MBP. We show that this intermediate is thermodynamically stable and retains maltose-binding ability. Forming the mechanically most stable structure within MBP, breakdown of this intermediate state occurs at ∼ 50 pN. Structurally, this unfolding event comprises the entire N-terminal domain of MBP. Clearly reflecting the two-lobe structure of MBP, the remaining C-terminal domain unfolds at ∼ 25 pN leading to the fully unfolded protein. Maltose-binding did not have a detectable effect on the unfolding kinetics of MBP in this pulling geometry. In different pulling directions, however, we show that maltose-binding does significantly change the force required to unfold the protein. This clearly demonstrates that in contrast to global thermodynamic stabilisation observable in bulk unfolding, ligand binding results in only local mechanical stabilisation when probed in single molecule force spectroscopy experiments.

Abstracts

– Single molecule mechanics –

P-12

Theoretical study of sequence dependent nanopore unzipping of DNA

U. Bockelmann, V. Viasnoff
Nanobiophysique, ESPCI, 10 rue Vauquelin, 75005 Paris, France

Nucleic acids can be driven through an individual nanopore formed by a heptamer of α-hemolysin reconstituted in a lipid bilayer. Applying a voltage U across the membrane leads to an ion current flowing through the pore. The α-hemolysin pore exhibits an open diameter of 1.8 nm. Therefore, only single stranded DNA or RNA can be threaded through. The ion current through the pore transiently drops during the passage of a nucleic acid. Molecular constructs featuring a duplex part preceded by a single stranded overhang can be threaded into the pore, transiently held in the pore at low voltage U and subsequently unzipped at higher U.
We explicitly take the DNA base sequence into account and theoretically study the nanopore unzipping of DNA. The process is described by a biased random walk in a one-dimensional energy landscape determined by the sequential basepair opening. Distributions of translocation times are numerically calculated as a function of duplex length, applied voltage und temperature. A rich dynamics is revealed for the coupled unzipping and translocation, bridging two different asymptotic regimes. One is a predominantly diffusive behaviour with a sequence specific effective diffusion constant. The other one is a dynamics dominated by pinning of the unzipping by rare events in the base sequence, namely the appearance of strong energy barriers to be thermally jumped over.
The work suggests experimental studies of sequence effects, by measuring the statistical distribution of unzipping times and comparing average values and mean-square deviations as a function of duplex length, bias voltage and temperature.

P-13

Single molecule protein folding experiments under force reveal a glassy energy landscape

J. Brujic, R. I. Z. Hermans, S. Garcia-Manyes, J. M. Fernandez
Columbia University, Biological Sciences Department, New York, USA

Ensemble studies of protein unfolding are typically modeled as two state reactions with a well-defined rate constant. Here we examine whether this view also holds at the single molecule level. We have developed force-clamp spectroscopy to follow the end-to-end length of single small proteins during their folding reaction. We first measure the kinetics of unfolding of the protein ubiquitin under a constant force and discover a surprisingly broad distribution of unfolding rates that follows a power law with no characteristic mean. The structural fluctuations that give rise to this distribution reveal the architecture of the protein's energy landscape. Following models of glassy dynamics, this complex kinetics implies large fluctuations in the energies of the folded protein, characterized by an exponential distribution with a width of 5-10 kbT. Our results predict the existence of a ``glass transition'' force below which the folded conformations interconvert between local minima on multiple time-scales. Both the unfolding as well as the folding pathways captured by force-clamp spectroscopy are much more complex than the two state model that is commonly used to interpret such data in classical protein biochemistry. Our results point to the necessity of using statistical mechanics to fully describe the folding of proteins under a stretching force (Brujic et al, Nature Physics, 2, 282-286, 2006).

P-14

Investigating non-processive molecular motors under constant force on the microsecond time-scale

M. Capitanio[1], D. Beneventi[1], M. Canepari[2], M. Maffei[2], R. Bottinelli[3], F. S. Pavone[1]
[1]LENS, Univ. of Florence, Italy, [2]Dept. of Experimental Medicine, Human Physiology unit, Univ. of Pavia, Italy, [3]Laboratory of Physiology, DBAG, Univ. of Florence, Italy

Many functions fundamental for cell life are performed by molecular motors, enzymes capable of converting the chemical energy contained in molecules like ATP into mechanical work and movement. Single molecule techniques have allowed directly investigating mechanical and biochemical states of single molecular motors. In particular, force-clamp methodologies have enabled interrogating how transitions between different conformations are affected by load. However, in current force-clamp techniques applied to non-processive motors force is not applied immediately after attachment of the motor to its track, but after a delay (4-5 ms) during which the motor protein goes through its working stroke. Moreover, the typical time resolution at which the protein mechanical states are sampled (100 μs) and the minimum duration of the detected events (5 ms) might hide some important feature within the motor cycle. Here, we developed a novel force-clamp assay for studying the interaction between a single myosin motor and an actin filament. A constant positive or negative force can be continuously applied to the actin filament, so that the delay between myosin binding and force application is completely abolished. Force in the range from 0 to $\sim\pm$12 pN can be applied. Data are acquired at sample intervals of 5 μs and events as short as 100-200 μs can be clearly detected due to the high signal-to-noise ratio of the method. The method can be applied to a wide range of non-processive molecular motors, single heads of processive motors, or ensemble of motor proteins.

P-15

Single molecule mechanics of cadherin interactions regulating cell-cell contacts

O. Courjean[1], E. Perret[2], S. Chevalier[1], H. Feracci[1]
[1]Université Bordeaux I, Centre de Recherche Paul Pascal, UPR CNRS 8641, Pessac, France, [2]Weill Medical College of Cornell University, M. Dyson Vision Research Institute, New York, USA

Classical cadherins are transmembrane glycoproteins involved in cellular adhesion. Adhesive interactions are achieved by their extracellular segment, but the molecular mechanism is still unclear and controversial. One of our aims is to probe their adhesive properties at the molecular scale and to better understand how dynamics regulates this mechanism (Perret et al., EMBO J. 2002; PNAS 2004). The Laminar Flow Chamber method was used as a very sensitive approach to study the kinetics parameters of E-cadherin interactions. Molecular interactions are visualized by studying the displacements of functionalized beads dragged by a laminar flow near a functionalized surface. By adjusting the nature and the density of various cadherin fragments on each surface, this technique allowed us to highlight the influence of single amino acids on binding formation and/or on stability of this interaction. Our studies strongly suggest an unexpected two-step mechanism by which one of both molecules could be sufficient to initiate the adhesive process. Moreover, the dissymmetrical intermediate complex could participate in the reinforcement of adhesive contacts by cadherin oligomerization. The influence of Ca2+ ions, necessary for the biologically active conformation of cadherins, on their adhesive properties under stress was also investigated. These biophysical studies provide a better understanding of the molecular processes used by cadherins for maintenance of cohesion and also plasticity of cell-cell adhesion.

Abstracts

– Single molecule mechanics –

P-16

Real-time imaging of DNA twisting properties of single Rad51 molecule

A. Dupont[1], H. Arata[2], J. Miné[1], M. Takahashi[3], J.-L. Viovy[1], G. Cappello[1]

[1]Institut Curie, Paris, France, [2]The university of Tokyo, Japan, [3]UMR 6204, CNRS and university of Nantes, France

Rad51 is the major eukaryotic protein involved in the homologous recombination repair of DNA double-strand breaks. The proccess is held in three steps: it first requires the formation of a helical nucleoprotein filament, which then aligns with the homologous DNA and finally proceeds to the strand exchange. Single molecule experiments and theoritical investigations have shown that both the polymerization of recombination proteins on the DNA and the strand invasion are associated with a strong variation of the DNA mechanical properties. In particular, DNA is stretched by a factor 1.5 and underwound to 18.6 base pairs per helical turn in the filament. While DNA elongation is well characterized experimentally using single molecule techniques as magnetic or optical tweezers, twisting and torque remain obscure at the molecular level. Here we report the real-time observation of twisting motion during polymerization of human Rad51 (hRad51) on dsDNA in a torque sensitive version of magnetic tweezers. Since this system realises a few degrees resolution, we observed stepping motion corresponding to the binding of single hRad51 during the elongation phase. The torque produced by hRad51 at maximum power was measured showing a higher value than the torque needed to denature DNA (but lower than the torque produced by the F1-ATPase). This real-time torque-sensitive apparatus reveals molecular details of the hRad51 polymerization on dsDNA resolving individual monomer binding and opens the possibility to explore the others steps of homologous recombination.

P-17

A Finite Elements Model of Microtubule Based on Molecular Dynamics Simulations

S. Enemark[1], M. A. Deriu[2], E. Votta[1], F. M. Montevecchi[2], A. Redaelli[1], M. Soncini[1]

[1]Dept. of Bioengineering, Politecnico di Milano, Milan, Italy, [2]Dept. of Mechanics, Politecnico di Torino, Turin, Italy

Microtubules (MTs) are part of the cytoskeleton of eukaryotic cells. Their mechanical properties depend on properties of the tubulin dimer, its interactions with surrounding dimers and geometric organization within MTs. While the geometry is well described in literature, the mechanical characteristics of the dimer and individual monomers are still undetermined. Such characteristics may help us understand the MT tensile properties on a smaller scale.

A mesoscale model of MT is constructed with a bottom-up approach. Using molecular dynamics (MD) simulations, elastic constants of the monomers are determined together with the interaction force between them. A 1 μm long MT was then constructed as a cylinder with interacting elastic elements, and its properties was examined via finite elements simulations.

The results show an elastic constant for α-tubulin and β-tubulin of 11 N/m and 15 N/m, respectively. For interactions between neighbouring monomers, the elastic constants along the protofilament (45 N/m for the intra-dimer interface and 18 N/m for the inter-dimer interface) are more rigid than elastic constants calculated for lateral interfaces (11 and 15 N/m). The mesoscale model provides mechanical properties of the whole MT and allows comparison with previous experiments and theoretical studies. We report a Young modulus of 1.66 GPa for the MT under axial tension. In perspective our approach provides a simple tool for analysing MT mechanical behaviour under different conditions.

P-18

Investigation of the molecular motor involved in malarial parasite invasion of erythrocytes

R. E. Farrow, J. A. Fielden, M. Grainger, J. L. Green, A. A. Holder, J. E. Molloy

MRC National Institute for Medical Research, London, UK

Myosins form a diverse superfamily of motor proteins capable of translocating actin filaments using the energy of ATP hydrolysis. *Plasmodium falciparum* myosin A (PfMyoA) is a class 14 myosin implicated in the invasion of erythrocytes by malarial merozoites. We have used an *in vitro* motility assay and optical trapping nanometry to investigate the properties of PfMyoA purified from merozoites. The purified myosin was adhered to a microscope coverslip using antibodies raised to the myosin tail interacting protein (MTIP). Fluorescence video microscopy was used to observe movement of rhodamine-phalloidin labelled actin filaments in the presence of Mg.ATP. We found that the sliding velocity was 3.5 μm.s^{-1}, similar to that of fast skeletal muscle myosins, and consistent with the speed at which the merozoite penetrates the erythrocyte. We are using an optical tweezers-based device to measure the force and movement produced by a single PfMyoA as it interacts with actin. A single, rhodamine-phalloidin labelled, actin filament is held between 2 latex microspheres and positioned so that intermittent interactions with a single PfMyoA molecule can be observed. These experiments enable the myosin power stroke to be measured directly. We are also attempting to express PfMyoA in a heterologous system, so that we can perform transient kinetic characterization of its ATPase pathway and study the effect of small molecule inhibitors on recombinant forms of the protein.

P-19

Single protein force spectroscopy using optical tweezers

J. C. M. Gebhardt, M. Rief

Technical University of Munich, James-Franck-Straße, 85748 Garching, Germany

Controlled application of force on proteins opens up a valuable source of information about the molecule of interest. As a single molecule approach, force spectroscopy allows the folding characteristics or conformational changes of individual proteins to be studied free from the blurring effects which occur in bulk measurements.

We constructed a mechanically stable optical tweezers apparatus ideally suited to probe the low force regime, up to 70 pN. With this device, two beads can be simultaneously trapped by two independently controllable beams. Force is transmitted from the beads to the protein via DNA handles. These are covalently connected to cystein residues in the protein using thiol-modified bases. Thus, the direction of force application can be arbitrarily varied. Using this setup, we study the unzipping behaviour of single coiled-coil proteins.

Abstracts

– Single molecule mechanics –

O-20

Applications of Single Molecule Recognition Force Microscopy

P. Hinterdorfer
Johannes Kepler University of Linz, Austria

We showed that two step unbinding events in single molecule force spectroscopy experiments between single-stranded DNA containing multiple methylcytosines and an anti-methylcytosine antibody can survey the distances between methylcytosines with single nucleotide resolution. Using different DNA sequences, the applicability for methylcytosine sequencing and the detection of single nucleotide polymorphism at the single molecule level was demonstrated. Secondly, simultaneous topography and recognition imaging (TREC) was applied to gently fixed microvascular endothelial cells in order to identify binding sites of vascular endothelial (VE)-cadherin. The recognition images revealed single molecular binding sites and prominent, irregularly shaped "dark" spots (domains) with sizes ranging from 10 to 100 nm that were subsequently assigned to topography features. TREC represents an exquisite method to quickly obtain the local distribution of receptors on cellular surface with an unprecendented lateral resolution of 5 nm.

O-21

Direct observation of equilibrium folding / unfolding transitions of single Calmodulins by AFM

J. P. Junker, M. Rief
Physik-Department E22, Technische Universität München, Garching, Germany

Single-molecule force spectroscopy by AFM allows investigating the unfolding and refolding kinetics of proteins. For the proteins studied so far with this technique, unfolding usually occurs far from thermodynamic equilibrium, i.e. the probability for refolding is negligible in the force regime necessary for unfolding. In equilibrium systems, such as overstretched DNA, polysaccharides or coiled-coil motifs, both unfolding and refolding takes place at rates generally too fast to observe individual transitions. Here we present data on the mechanical unfolding of Calmodulin which occurs close to equilibrium. Numerous folding/unfolding transitions can be observed in the traces while stretching the protein at extremely low pulling velocity (1 nm/s). This data yields direct access to the unfolding and refolding rates under force and will allow a detailed mapping of the potential energy surface for the mechanical unfolding and refolding of the protein. The two globular domains of Calmodulin unfold independently at forces <20 pN and refold at $\sim$10 pN. Calmodulin binds 4 calcium ions and accomplishes a large conformational transition upon binding of a target peptide such as the wasp venom peptide Mastoparan. Our experimental data shows that ligand binding clearly increases the mechanical stability of the protein and modulates the unfolding and refolding rate. Moreover, binding of Mastoparan does not require complete folding of both Calmodulin domains simultaneously. The influence of ligands on the unfolding and refolding rate of Calmodulin becomes directly observable in great detail.

O-22

Motility and regulation of the tetrameric mitotic kinesin Eg5

L. C. Kapitein[1], B. H. Kwok[4], S. Lakämper[2], M. J. Korneev[3], S. Reiter[2], T. M. Kapoor[4], E. J. Peterman[1], C. F. Schmidt[2]
[1]Dept. Physics and Laser Centre, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands, [2]III. Phys. Inst., Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany, [3]ASML, De Run 6501, 5504 DR Veldhoven, The Netherlands, [4]Lab. Chem. Cell Biol., The Rockefeller University, New York, NY 10021, U.S.A.

Tetrameric kinesin-5 motor proteins are needed for eukaryotic cell division. We have shown that the kinesin5 Eg5 can slide microtubules apart, and we have studied it's processivity and regulation by the drug monastrol. Furthermore we have investigated the physiological regulation of this motor in optical trapping and single-molecule fluorescence assays. Assembly and maintenance of the spindle is a highly controlled process. Some kinesins have been found to be cargo-activated, but for a tetrameric motor such was Eg5 it is not obvious how a corresponding mechanism could function. We have examined factors that influence the switching of Eg5 between a directional and a diffusive mode, varying buffers and microtubule-binding geometries. We found that at moderate ionic strength, Eg5 moves directionally. In contrast, at higher ionic strength Eg5 diffuses along microtubules without directional bias. Remarkably, under these conditions Eg5 still moves directionally when bound between two microtubules. In the spindle, this functional specialization might allow Eg5 to diffuse on single microtubules without hydrolyzing ATP until the motor gets activated by binding another microtubule.

P-23

Zooming-in on kinesin´s stepping

Z. Lansky, S. Verbrugge, E. J. G. Peterman
Department of Physics and Astronomy and Laser Centre, VU university Amsterdam, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

Kinesin-1 is a motor protein that walks processively along microtubules in a hand-over-hand manner driving intracellular transport of vesicles and organelles. The center of mass of the motor moves in steps of 8 nm per ATP hydrolyzed, each step taking approximately 10 ms at saturating ATP concentrations. Many details of the molecular mechanism of kinesin's stepping process are still not fully understood, mainly due to limitations of the widely applied single-molecule methods. Traditional wide-field single-molecule studies suffer from a limited temporal resolution, while it is impossible to directly observe conformational changes of a single motor domain using optical tweezers. Here, we use a novel method based on confocal fluorescence microscopy to characterize Kinesin-1's stepping process. In our approach a laser beam is focused on an immobilized microtubule and the photons emitted by the fluorescently-labeled motors walking through the focus are collected, detected and time-tagged with nanosecond accuracy. In this way we can resolve fluorescence intensity fluctuations on a timescale of $\sim 10\mu$s. We apply this approach to kinesin constructs that are labeled with a donor fluorophore on the one motor domain and an acceptor on the other and we follow the conformational changes of the kinesin molecule during the stepping process by Förster resonance energy transfer between the two dyes. Our first experiments are in agreement with models in which both motor domains are 8 nm apart, bound to successive tubulin dimers for most of the time, while stepping is faster than 200 microseconds.

Abstracts

– Single molecule mechanics –

P-24

Detection of fractional steps in cargo movement by the collective operation of kinesin-1 motors

C. Leduc, F. Ruhnow, J. Howard, S. Diez
MPI-CBG, Dresden, germany

The stepping behaviour of single kinesin-1 motor proteins has been studied in great detail. However, in cells, these motors often do not work alone but rather function in small groups when they transport cellular cargo. Until now, the cooperative interactions between motors in such groups are poorly understood. A fundamental question is whether two or more motors that move the same cargo step in synchrony, producing the same step size as a single motor, or whether the step size of the cargo movement varies. To answer this question, we performed *in vitro* gliding motility assays where microtubules coated with quantum dots were driven over a glass surface by a known number of kinesin-1 motors. The motion of individual microtubules was then tracked with nanometer precision. In the case of transport by two kinesin-1 motors, we found successive 4-nm steps, corresponding to half the step size of a single motor. Dwell-time analysis did not reveal any coordination - in the sense of alternate stepping - between the motors. When three motors interacted in collective transport we identified distinct forward and backward jumps in the order of 10 nm. The existence of the fractional steps as well as the distinct jumps illustrate a lack of synchronization and has implications for the analysis of motor-driven organelle movement investigated *in vivo*.

P-25

Stiffness of the myosin head and lifetime of the actomyosin rigor bond under strain

A. Lewalle[1], W. Steffen[2], O. Stevenson[1], Z. Ouyang[1], J. Sleep[1]
[1]Randall Division, King´s College London, London SE1 1UL, United kingdom, [2]Medical School Hanover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

The force-extension curve of a myosin subfragment-1 molecule when interacting in the rigor state with an actin monomer has been investigated by moving a bead-actin-bead dumbbell held in optical tweezers past a myosin at low [ATP]. It was found to be linear in the physiological range of force ($\pm$ 10 pN). The stiffness of the myosin head was about 2 pN/nm, a value consistent with measurements made at the same time using an earlier method based on the covariance of bead positions. The value is also consistent with the most recent report (1.7 pN/nm) for a rabbit myosin head in a muscle fibre (Linari et al, Biophy. J. **92**, 2476). We observe an asymmetry in the strain dependence of the rate of dissociation of myosin from actin, with the rates being greater for forces that resisted the myosin working stroke.

P-26

Force induced unfolding of G-quadruplex studied by molecular dynamics

H. Li[1], E. Cao[2], T. Gisler[1], G. Maret[1]
[1]Department of physics, University of Konstanz, 78457 Konstanz, Germany, [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

DNA oligonucleotides composed G repeat sequences can adopt G-quadruplex structures in the presence of specific metal ions, such as Na^+, K^+. Because of their importance in *vivo* and in *vitro*, numerous studies have been done about the structure and stability of the G-quadruplex. AFM or optical tweezers provides a novel method to study the stability of DNA or protein in single molecular lever by force-induced unfolding. These experiments measure the force required to stretch or unfolding a molecular domain. However, it is not possible yet to relate the measured force with the changes of structure.
Here, we used molecular dynamic simulation to study the force induced unfolding of G-quadruplex (sequence 5'-GGGGTUTUGGGGTTTTGGGGUUTTGGGI-3'). The unfolding process was followed and the force required were measured depend on the stretching parameters (pulling velocity and stiffness of cantilever) and environment (ion category and concentration). The free energy changes were also calculated by perturbation.

P-27

Torque, speed, sodium-motive-force and steps of chimeric Bacterial flagellar motor

C.-J. Lo, M. C. Leake, Y. Sowa, R. M. Berry
Clarendon Laboratory, Department of Physics, University of Oxford, Parks Road, Oxford, UK.

Many species of bacteria swim by means of flagella, a long helical filament connected to rotary molecular motors embedded in the cell membrane. Motors can run up to several hundred hertz and propel the cell body up to 30 μm/s. The motor contains parts from both proton- and sodium-driven motors and is driven by sodium ion flux and sodium-motive force (smf), comprising membrane potential (V_m) and sodium concentration gradient (ΔpNa) across membrane. We have developed single bacterial cell fluorescence measurement of intracellular sodium concentration ($[Na^+]_{in}$) and V_m to measure the smf of the chimeric motor. Combining high resolution single motor speed measurements and a fast flow-cell, we study the motor speed and functions in various regimes of load, number of torquegenerating units and driving force. We measured the torque-speed relationship and the speed response to the driving in different load. Direct observations of steps in stable rotation and dwell time distributions are also presented.

Abstracts

– Single molecule mechanics –

P-28

Studying molecular motors using total internal reflection fluorescence microscopy

C. Mellor[1], M. R. Webb[1], J. L. Hunter[1], S. Toba[2], K. Oiwa[2], J. E. Molloy[1]

[1]MRC National Institute for Medical Research, London, UK, [2]National Institute of Information and Communications Technology, Kobe, Japan

Kinesins and dyneins are microtubule-based molecular motors that convert chemical energy derived from ATP hydrolysis to force and movement. We have studied the properties of an axonemal dynein called dynein-f (or dynein I1), using Total Internal Reflection Fluorescence (TIRF) microscopy. TIRF microscopy utilises the evanescent field created by a totally internally reflected laser beam to illuminate fluorophores at the surface of a microscope coverslip. The technique gives very high signal-to-noise ratio and allows single fluorophores to be observed at the surface. Our aim has been to measure the stepping pattern of an individual dynein-f as it walks along a single microtubule (MT) using nanometre tracking of the fluorescence image. We have validated our system using conventional kinesin labelled with a single quantum dot (QD) and found that its stepwise motion along the MT consists of discrete 8 nm jumps. We are currently analysing data obtained using QD-labelled dynein-f to determine if its motion also exhibits regular steps. We are also developing a single molecule chemical probe, to measure the production of phosphate during motor activity. The protein-based, phosphate sensor is immobilised on the microscope coverslip and generates a fluorescence signal as it binds individual phosphate ions. We aim to combine this sensor with our *in vitro* assays of motor function so that we can correlate motor movement with ATPase activity.

P-29

Torsional behaviour of human Rad51 nucleoprotein filaments using single molecule experiment

J. Miné[1], L. Disseau[1], T. Masayuki[3], M. Dutreix[2], J.-L. Viovy[1]

[1]Institut Curie, CNRS UMR 168, Paris, France, [2]Institut Curie, CNRS UMR 2027, Orsay, France, [3]Nantes University, CNRS UMR 6204, France

Homologous recombination is an essential biological process to repair double strand breaks of DNA: it involves the pairing and exchange between two homologous DNA molecules. Understanding and controlling homologous recombination is of the upmost importance for medical purposes because of its implication in cancer. In eukaryotes, Rad51 protein is the central enzyme involved in homologous recombination.

We use magnetic tweezers to study homologous recombination at the single molecule level. It enables us to exert both a stretching force in the pN range, a torsional constraint on a single DNA molecule, and to measure its extension in real time with a precision of 10 nm. Because double stranded DNA molecules are usually topologically constrained *in vivo*, torsional constraints that affect DNA, and thus DNA-protein interactions, are of particular interest. In this study, we characterized the mechanical and torsional behaviour of human Rad51-DNA fibers. We showed that unwinding a dsDNA induced a fast hRad51 polymerisation and that hRad51 polymerisation on double stranded DNA is coupled to supercoiling. Moreover, we observed that once a hRad51 fiber is formed, applying positive turns do not induce hRad51 depolymerisation.

P-30

Real time study of nucleation, growth and dissociation of single Rad51-DNA nucleoprotein filaments

J. Miné[1], L. Disseau[1], M. Takahashi[2], G. Cappello[1], M. Dutreix[3], J.-L. Viovy[1]

[1]Institut Curie, Paris, France, [2]CNRS and University of Nantes, Nantes, France, [3]Institut Curie, Orsay, France

Homologous recombination consists in a strand exchange between two homologous DNA molecules. It plays a fundamental role in DNA repair and genome maintenance. Rad51 protein is the central enzyme involved in this process in eukaryotes. *In vitro*, it interacts with both dsDNA and ssDNA, forming helical nucleoprotein filaments. As a first step to understanding the whole recombination process, we study the polymerization and depolymerization of human Rad51 on single DNA molecules, using magnetic tweezers. These tweezers enable us to exert a stretching force in the pN range and to measure its extension in real time with a 10 nm resolution. The dependence of the initial polymerization rate on dsDNA upon hRad51 concentration indicates that the rate limiting step is the formation of a nucleus involving 5.5 ± 1.5 hRad51 monomers, corresponding to one helical turn of hRad51. The kinetic polymerization profile presents several regimes depending on protein concentration, associated with a change in the balance between nucleation and growth. Our results also confirm that, from a thermodynamic point of view, the favorite substrate of hRad51 is dsDNA. However, we also demonstrate that hRad51 polymerizes faster on ssDNA, and that its thermodynamic preference for dsDNA is entirely due to a depolymerization rate 150 times slower than from ssDNA. All these features contrast with the behavior of RecA, hRad51's bacterial homologue, and suggest that an essential role of hRad51 partner proteins could be to facilitate its depolymerization from dsDNA.

P-31

Biophysical characterization of the p73 protein

S. Patel, A. Mmerabet

Kings College, London, U.K.

The p53 protein family has fascinatingly complex biological functions and is involved in the control of an intricate network of genes implicated in DNA repair, growth arrest, apoptosis, cell proliferation, germ line integrity, development and metabolism. While the role of p53 as a guardian of the genome is well established, the intrinsic nature of p73 is yet to be fully elucidated. The discovery of p63 and p73 has raised many questions about their intrinsic and collective functions. Specifically, the structural and molecular studies so far have been focused on p53, and to a degree on p63, while p73 is poorly characterized. Here we present biophysical characterization of the p73 protein and examine the implications for its biological function(s), cancer and its interplay in the p53 network. In contrast to p53, the monomeric DNA binding domains of p73 and p63 did not bind to 30-mer gadd45 dsDNA, as tested by fluorescence anisotropy. However, a larger p73 DBD+TET construct did bind gadd45. Significantly, the p73 DBD exhibited enhanced thermodynamic stability relative to the p53 DBD as shown by Differential Scanning Calorimetry, DSC, Circular Dichroism, CD, and equilibrium unfolding, but not compared to p63 DBD. The p73 DBD+TET is less stable than p73 DBD as shown by DSC, CD and equilibrium unfolding. These stability and binding data for p73 enhances our current understanding of the role of the p73 protein in the evolution of tumor suppression and cancer.

Abstracts

– Single molecule mechanics –

P-32

Myosin V stepping mechanism studied by travelling wave tracking

P. Pierobon[1], G. Cappello[1], C. Symonds[1], L. Busoni[1], J. C. M. Gebhardt[2], M. Rief[2], J. Prost[1]

[1]Institut Curie, CNRS UMR 168, 11 rue P. et M. Curie, F-75231 Paris Cedex 05, France, [2]Physik-Department E22, Technische Universität München, James-Franck-Strasse, D-85748 Garching, Germany

We perform bead assay experiments to observe Myosin V stepping by Travelling Wave Tracking. This technique, combining nanometer accuracy with microseconds temporal resolution, allows one to track the particle in the two directions X and Z, respectively parallel and perpendicular to the Myosin V motion. Upon averaging on many steps with different concentration of ATP we identify three phases in the well known 36nm step: a first 5nm substep (the typical time scale of which depends on the ATP concentration), a fast motion to 25nm followed by a diffusional search toward the next binding site. Based on these data we propose some hypothesis on the mechano-chemical cycle of Myosin V.

P-33

Mechanical oscillations of an actin filament driven by a collection on non processive motor proteins

P.-Y. Plaçais, M. Balland, P. Martin

Institut Curie, Paris, France

Hair cells from the inner ear of the vertebrates can power spontaneous oscillations of their mechanosensory hair bundles, thereby enhancing their sensitivity to small stimuli at frequencies close to that of the spontaneous oscillation. A mechanism proposed to explain the oscillatory instability involves, in each stereocilium of the bundle, a group of non processive myosins (myosin 1c) that exert force on a mechanosensitive transduction channel and are regulated by calcium ions flowing into the cell when the channel opens. We designed in vitro experiments to determine by which mechanism calcium might affect the mechanical properties of these motors. We first showed that it was possible to get oscillations with a simple mimetic system including only HMM from myosin II, actin, and an elastic restoring force opposing the force exerted actively by the motors. In order to unveil the precise role of calcium in the hair bundle, we designed an experimental setup to perform single molecule experiments in a "3-bead assay" while simultaneously and dynamically changing the local calcium concentration by laser photolysis of caged Ca2+.

P-34

Single molecule studies on the mechanical activation of the molecular force sensor Titin Kinase

E. Puchner, H. E. Gaub

LMU Munich, Chair for Applied Physics, Biophysics & Molecular Materials, Amalienstr. 54 / 1, 80799 Munich, Germany

Molecular force sensors are the core of signaling pathways monitoring mechanical units or reporting mechanical load. It has been shown, that titin, a giant protein located in muscle sarcomers, contains a kinase domain that controls muscle gene expression and protein turnover. This location is ideal to sense the local load applied to the muscle. Molecular dynamics simulations suggest, that the titin kinase can be mechanically converted from its autoinhibited conformation into an active one with accessible ATP binding site.
We investigated the mechanical properties of titin kinase be means of single molecule force spectroscopy. Our results show, that this enzyme complies with the requirements of a force sensor. On the one hand the unfolding and activation forces are lower than the ones of the surrounding titin domains. On the other hand, the energy barriers are low enough to be overcome so that activation could take place. Re-enacting the natural setting on a single molecule level evidences, that during a mechanical activation cycle ATP is able to bind to the titin kinase.
Our results allow new insight into the function and dynamics of this intriguing enzyme and characterize the first, and until now missing part of this complex mechanical signaling pathway.

P-35

A kinetic model of muscle contraction

E. N. Shvorina

Institute of Mechanics, Moscow University, Russian Federation

Quantitative description of molecular mechanical processes underlying muscle contraction is an old problem of biophysics. Tension produced by contracting muscle results from cyclic interaction of myosin cross-bridges and actin filaments which is powered by ATP hydrolysis. Although a number of kinetic models of muscle contraction have been suggested during last 50 years, some of experimental observations cannot be reproduced by existing models. A kinetic scheme of the actin-myosin-ATP interaction in muscle includes several attached and detached states of cross-bridges and the rates of their transitions from one state to another. The number of the states, graph of possible transitions between them and the strain dependence of the rates vary in different models so that a flexible computer tool that enables one to change and tune the model easily could be very useful.
We are building a software complex that allows a flexible change of the kinetic model during simulation of mechanical and biochemical properties of contracting muscle fibres. Some blocks of the software were tested on a recent kinetic model containing 7 attached and 3 detached cross-bridge states (Ferenczi *et al*., Structure, 2005). The model describes quite well the dependence of fibre tension, stiffness and ATPase rate on temperature and concentrations of ATP, ADP and inorganic phosphate during steady-state isometric contraction. Simulations of experiments with steady-state shortening and lengthening of muscle fibres are on the way. A program block for simulation mechanical and biochemical responses to length and force step changes is under development.

Abstracts

– Single molecule mechanics –

P-36

Vibrational excited states and protein conformational changes

P. A. Silva, L. Cruzeiro
CCMAR and FCUL, University of Algarve, Faro, Portugal

Proteins can act like molecular machines converting chemical energy into work. The energetics of these processes is related with two main questions: (i) in what form is the energy released by the hydrolysis of ATP stored in the protein (and how does the system prevent its loss to the environment)? (ii) what is the mechanism for the conversion of that energy into usefull work? In the 1970's it was suggested that Vibrational Excited States (VES), in particular the vibrational mode known as Amide I, can efficiently keep the energy in the protein, thus providing a possible answer to the first question. The development of this idea (Davydov – Scott model) lead to the conclusion that Amide I excitations can be transferred from site to site in the protein, even at biological temperatures, in tens of picoseconds. Experimental results indicate that the energy of Amide I excitations indeed remains for tens of picoseconds in real proteins and model systems but not in its initial form. It is transferred to different vibrational modes. The referred model lacks a mechanism for this transfer because it conserves the number of Amide I excitations. An answer to the question (ii) stated above requires a non number conserving generalization of the model.
In this work we propose a non – conserving model in which: (i) the energy is stored and travels along the protein in the form of Amide I excitations; (ii) the excitations **site selectively** promote the disruption of structural constraints, such as the hydrogen bonds that stabilize the structure of proteins.
The Fundação para a Ciência e a Tecnologia is acknowledged for a Fellowship to P.A.S. Silva.

P-37

Exploring the free energy landscape of protein-protein association using BD simulations

A. Spaar[1], R. R. Gabdoulline[2], R. C. Wade[2], V. Helms[3]
[1]Consorzio Mario Negri Sud, Santa Maria Imbaro (Chieti), Italy, [2]EML Research GmbH, Heidelberg, Germany, [3]Center for Bioinformatics, Saarland University, Saarbrücken, Germany

To study the free energy landscape for protein-protein association, we performed Brownian dynamics (BD) simulations using a novel analysis, where the individual protein conformations are recorded along the BD trajectories and stored in occupancy maps. These maps can be interpreted as a probability distribution, which allows the calculation of the entropy landscape. From the resulting free energy landscape, we deduced details of the association process like the optimal association pathways and the encounter complex. We applied this analysis to study the association of barnase to barstar, a well characterized, electrostatically steered model system, and of cytochrome *c* to membrane-bound cytochrome *c* oxidase (COX). For the first system, we found that for low and medium ionic strength, a small free-energy barrier divides the energetically most favorable region into a region of the encounter complex and a region near the RNA binding loop. However, single mutations may drastically change the free-energy landscape and thus alter the population of the two minima. For the second system, we investigated the effect of the membrane on the association behavior. As expected, the optimal association pathways largely overlapped for cytochrome *c* associating towards soluble COX and COX embedded in an uncharged membrane. Remarkably, after switching on the lipid partial charges, cytochrome *c* is strongly attracted by the inhomogeneous charge distribution of the lipid head groups and shows preferential diffusion parallel to the DPPC membrane surface.

P-38

Shear-induced unfolding triggers adhesion of vWf fibers

D. M. Steppich[1], S. Nuschele[1], J. I. Angerer[1], S. W. Schneider[2], A. Wixforth[1], A. A. Katz[3], R. R. Netz[3], M. F. Schneider[1]
[1]University of Augsburg, Germany, [2]University of Muenster, Germany, [3]Technical University of Munich, Germany

Von Willebrand factor (VWF), a protein present in our circulatory system, is necessary to stop bleeding under high shear stress conditions as found in small blood vessels.
The results presented here, help unravel how an increase in hydrodynamic shear stress activates VWF's adhesion potential, leading to the counterintuitive phenomena of enhanced adsorption rate under strong shear conditions. Using a novel microfluidic device we are able to mimic a wide range of blood-flow conditions and directly visualize the conformational dynamics of this protein under shear-flow. In particular, we find that VWF displays a globule-stretch transition at a critical shear rate $\dot{\gamma}_{crit}$ away from any adsorbing surface. In the presence of an adsorbing collagen substrate, we find a large increase in the protein adsorption at the same critical shear rate. This suggests that the globule unfolding triggers the surface adsorption. Monitoring the adsorption process of multiple VWF fibers, we are able to follow the formation of an immobilized network, which constitutes a "sticky" grid necessary for blood platelet adhesion. Computer simulations reproduce this sharp transition, and identify the large size of VWF's repeating units as one of the keys for this unique hydrodynamic activation.
Since areas of high shear stress coincide with a higher chance for vessel wall damage by mechanical forces, we identify the shear induced increased binding probability of VWF as an effective self-regulating repair mechanism of our microvascular system.

P-39

The first fully automated single-molecule force spectroscopy system to screen molecular interactions

J. Struckmeier[1], R. Wahl[1], L. Riemenschneider[1], L. Amsel[1], H. Janovjak[3], A. Kedrov[2], D. J. Müller[2]
[1]nAmbition GmbH, Dresden, Germany, [2]Technical University, Dresden, Germany, [3]University of California, Berkeley, U.S.A.

Single-molecule force spectroscopy (SMFS) based on the atomic force microscopy is on the way to become an established technique for quantified analysis of intra- and intermolecular interactions.
Within the past decade SMFS provided substantially new insight into interactions that stabilize proteins, DNA, polymers or act between receptors and ligands. Recently it could be shown, that the extremely high sensitivity of the SMFS allows locating ligand or inhibitor binding to a membrane protein. Additionally it was shown, that the SMFS data can identify the functional state of a membrane protein. Thus, SMFS can be easily applied to study drug-target interactions such as required in pharmacology, cell biology or medical research and application. However, since SMFS is a single molecule technique it requires substantial efforts to gather statistically relevant data sets.
We will discuss the challenges in automation and standardization of the SCFS experiments and will present a fully automated SCFS system which sets new standards in data quality and data throughput. The enormous amount of recorded SMFS data is fully automatically processed and pre-analyzed.
Additionally the force resolution of the SMFS, which is limited by the Brownian motion of the cantilever, can be enhanced by photon driven active noise cancellation feature of the force sensing device.

Abstracts

– Single molecule mechanics –

O-40

AFM force spectroscopy of braided DNA

J. W. Strzelecki[1], A. Balter[1], P. E. Marszalek[2]
[1]Nicolas Copernicus University, Department of Physics, Torun, Poland, [2]Duke University, Center For Biologically Inspired Materials and Material Systems, NC, USA

Recent experiments with optical and magnetic tweezers and Atomic Force Microscope (AFM) enabled us to view single biomolecules as objects, that can be described not only by quantum mechanics, but also classical mechanics. These instruments enable us to determine mechanical properties of proteins, nuceic acids and polysacharides which are essential to better understand transcription and DNA – protein interactions.Previous experiments on DNA elasticity gave us much better understanding of this remarkable molecule. Howether, most of them were performed on linear DNA, while it always has supercoiled/chromatin fiber form in cells. Experiments on braided DNA with magnetic tweezers, while allowing to braid DNA molecules, did not allow stretching due to small pulling forces. In order to avoid this problem we use AFM and pull a braided structure that appears naturally in bacterial plasmids.
Thus, we perform an AFM pulling of supercoiled pUC18 plasmid to determine influence of braiding on DNA force extension characteristics. Additional control experiments with linearized DNA and lambda phage were also performed. We observed, that B-S transition plateau in stretched supercoiled plasmid appears at higher force (>250 pN) than in case of two DNA molecules stretched in parallel (203 pN). We think that this increase of overstretching force is caused by pulling of braided structure present in plasmids. We suggest that additional force observed comes from countering a hydration force repulsion of negatively charged backbones, forced into contact as the braid is being stretched.

O-41

Myosin V crossing Arp2/3 junctions: Predictions from the elastic lever arm model

A. Vilfan
J. Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

Myosin V is a two-headed processive motor protein that walks in a hand-over-hand fashion along actin filaments. Here we study the behaviour of a myosin V molecule encountering an actin junction formed by the Arp2/3 complex.
Our theoretical study is based on the elastic lever arm model for myosin V [1,2], which describes the dimeric molecule as two identical heads, connected with elastic lever arms. The geometry of a head in each chemical state has been determined from EM studies. We have shown previously that the model provides an explanation for the coordinated hand-over-hand motion and that it fits well the available force-velocity relations. To study the branching probability at Arp2/3 junctions, we calculate the shapes and bending energies of all relevant configurations in which the trail head is bound to the actin filament before Arp2/3 and the lead head is bound either to the straight or to the side branch. We assume that the probability that the lead head binds to a certain actin subunit is proportional to the Boltzmann factor obtained from the elastic energy. With this assumption our model predicts that the probability that a molecule turns onto the side branch is about 20% and the probability that it continues along the same filament 80%. This result is in good agreement with recent experimental data [3].
[1] A. Vilfan, *Biophys. J.* **88**, 3792-3805 (2005).
[2] A. Vilfan, *J. Chem. Inf. Model.* **45**, 1672-1675 (2005).
[3] M.Y. Ali *et al*., *Proc. Natl. Acad. Sci. USA* **104**, 4332-4336 (2007).

Abstracts

– RNA structure & function –

O-42

Structure and function of human telomerase

D. Alves, R. Codrington, H. Li, A. Orte, X. Ren, D. Klenerman, S. Balasubramanian
University Chemical Laboratory, University of Cambridge, Cambridge CB2 1EW, U.K.

Human telomerase is a ribonucleoprotein complex that comprises an RNA component (hTR) and a protein reverse transcriptase component (hTERT). Telomerase is responsible for the synthesis of single stranded telomeric DNA repeats (GTTAGG) at the telomeres, which serve to protect the ends of the chromosomes. The telomerase enzyme complex is a rather unusual DNA polymerase in that it self-templates the sequence of the telomeric repeats and furthermore, both the RNA and protein components are catalytically functional. We have studied the functional telomerase complex using single molecule biophysical methods. In particular, we have employed a methodology that uses two-colour coincidence detection (TCCD) to characterise complex formation between labelled biomolecules in solution. By systematic labelling and in vitro assembly of hTERT, hTR and telomeric DNA substrate components, we have been able to establish the composition of the human telomerase core particle. The details of this study will be presented along with an update of our other experiments on telomerase function using single molecule FRET.

O-43

Equilibrium/non-equilibrium transition in biological macromolecule interactions

P. Dumas, S. Bernacchi, E. Ennifar
IBMC-CNRS & Univ. Louis Pasteur, Strasbourg, France

From the particular problem of "melting studies", used extensively with nucleic acids and proteins, we were led to the general problem of the transition between equilibrium and non-equilibrium upon temperature variation. Numerical simulations on a simple system predicted a hysteresis phenomenon, which is the hallmark of departure from equilibrium. Considerations on the "relaxation time" of a system allowed to define a critical value T_r of the temperature separating equilibrium from non-equilibrium regimes. It is predicted that the transition between the two regimes may be very sharp and (almost) concentration-independent. The critical, or "relaxation temperature" T_r, depends on the heating rate dT/dt according to $\Delta(1/T_r) = -(R/E_{off})\,\Delta(Ln\ dT/dt)$, E_{off} being an activation energy (*e.g.* for duplex dissociation). This allows to derive E_{off} by monitoring the variation of T_r with dT/dt. Also, T_r may be expressed as the ratio $\Delta H_r/\Delta S_r$ of enthalpic and entropic terms, which extends to non-equilibrium the thermodynamic relationship $T_m = \Delta H/\Delta S$ defining a melting temperature T_m. Contrary to a common intuition, T_r never becomes an approximation for T_m when $dT/dt \rightarrow 0$. These results were experimentally verified with melting studies on short nucleic acids able to form hairpins and duplexes. They can readily be extended to proteins and Differential Scanning Calorimetry. Several examples from the literature also allowed verifying our results. They might shed light on recent advances in the field of RNA-mediated genetic control, particularly for thermosensor RNAs directing the fate of a bacterium on temperature change.

P-44

Structural and functional studies of hirsutellin A: a new ribotoxin.

E. Herrero-Galán, J. Lacadena, Á. Martínez del Pozo, M. Oñaderra, J. G. Gavilanes
Department of Biochemistry and Molecular Biology I, Faculty of Chemistry, Complutense University, Madrid, Spain

The cytotoxicity of ribotoxins is related to the inactivation of ribosomes by a specific ribonucleolytic action against a highly conserved sequence of the 28S ribosomal RNA, resulting in protein biosynthesis inhibition. The three dimensional structures of the most representative members of the ribotoxins family (α-sarcin and restrictocin) have been elucidated. However, knowledge about the structural elements responsible for their specific ribonucleolytic action and their ability to cross membranes is scarce. These proteins also display some sequence and three-dimensional structure similarities to other non-toxic fungal RNases such as RNases T1 and U2. They differ in length and arrangement of the protein loops of the non periodic structure.

Hirsutellin A (HtA) shows biological properties similar to those of the α-sarcin family. However, HtA displays only 25% sequence identity with α-sarcin. HtA is 20 residues shorter and structural differences in the arrangement of the protein loops and N-terminal β –hairpin are predicted. Our studies are directed towards the identification of the structural elements responsible for the differences between ribotoxins and the other non toxic RNases. HtA exhibits specific ribonucleolytic activity and it has been observed to be cytotoxic to rhabdomyosarcoma cells, but the nature of its interaction with phospholipid membranes is different from that of α-sarcin. The results are discussed in terms of ribotoxins evolution and structure-function relationships in this family.

P-45

Probing the flexibility of SL1 kissing complex from HIV-1 genomic RNA by MD simulation

S. Mazier, D. Genest
CBM, CNRS Orléans France

The SL1 stem-loop located in the encapsidation domain is responsible for initiating the dimerisation of HIV-1 genomic RNA by means of a loop-loop interaction known as Kissing Complex (KC). The SL1 secondary structure has been predicted as a 35 nucleotides stem-loop composed of a 4 base pairs (bp) terminal duplex , a 4 nt asymmetrical internal loop, a 7 bp internal duplex and a 9 nt apical loop. Several high resolution structures of the monomer and of KC of a 23 nt sequence containing only the internal duplex and the apical loop of SL1 are available in the literature. No experimental high resolution structure of the complete native SL1 sequence has been reported so far, neither for the monomer nor for KC. The asymmetrical internal loop has been described from NMR studies of different monomeric hairpin sequences, leading to divergent results which suggests its high flexibility. In this work, we built a $SL1_{35}$ KC model which was submitted to a 31 ns molecular dynamics simulation (MD).

Our results allows to describe the internal dynamics of $SL1_{35}$ KC and the differences of behaviour of the different parts of the dimer. Thus, we could show the stability of the interactions between the 2 apical loops and of the terminal duplexes, the destabilisation of the internal duplexes and the high flexibility of the asymmetrical internal loops.

Abstracts

– RNA structure & function –

O-46

Understanding gene regulation from the NMR structures of several protein-RNA complex

F. Oberstrass, S. Auweter, Y. Hargous, L. Skrisovska, F. Allain
Institute for Molecular Biology and Biophysics, ETH Zurich, Switzerland

Gene regulation at the posttranscriptional level is controlled by a multitude of mRNA binding proteins. These proteins recognized often the RNA nucleotide sequence of their mRNA targets but also sometimes their shape. We have recently determined the solution structures of several proteins in complex with RNA. Some (PTB, Fox, SRp20, hnRNP F) binds single-stranded RNA recognizing the RNA sequence while others like VTS1 or RBMY binds an RNA stem-loop recognizing rather its shape.
The single-stranded RNA binding proteins are all involved in the regulation of alternative-exons and all contain one (Fox and SRp20), three (hnRNP F) or four copies (PTB) of the most common RNA Recognition Motif (RRM). Although RNA binding involved the same type of domain, the affinity and mode of RNA recognition varies widely between the different proteins. We will discuss how these different modes of recognition help understanding the specific functions of the different proteins.
The stem-loop recognitions by Vts1p (the yeast homologue of the *Drosophila* Smaug) and by RBMY are also very different. While the SAM domain of Vts1p recognizes predominantly the shape of the pentaloop rather than its sequence, the RRM of RBMY recognizes the shape of the stem and the sequence of the pentaloop. Complex formation with RNA involves a rigid-body-fit for VTS1p while it requires an induced-fit for RBMY.
Altogether, the very diverse modes of RNA recognition by small protein domains correlate well with the many different gene regulation processes controlled by RNA-protein interactions.

O-47

The structural basis of ribozyme-catalyzed RNA assembly and its implications for an RNA world

M. P. Robertson, W. G. Scott
Center for the Molecular Biology of RNA, University of California at Santa Cruz, U.S.A

Life originated, according to the RNA World hypothesis, from self-replicating ribozymes that catalyzed the ligation of RNA fragments or monomers. We have solved the 2.6Å crystal structure an in vitro-selected RNA ligase ribozyme that catalyzes the regiospecific formation of a 5'-3' phosphodiester bond between the 5'-triphosphate and the 3'-hydroxyl termini of two RNA fragments in a reaction that mimics that of cellular RNA polymerases. A network of invariant nucleotides form tertiary contacts that stabilize a flexible stem of the ribozyme in proximity to the ligation site. A resulting inter-helical base triple positions two separate sets of conserved residues together to form a compact catalytic pocket. A magnesium ion at the ligation site coordinates three phosphates, bridging the two helices while apparently activating the ligation site phosphate for catalysis. The structure of the active site permits us to suggest how transition-state stabilization and a general base may catalyze the ligation reaction. RNA-catalyzed RNA polymerization is the foundation of the RNA World hypothesis and this structure offers the first high-resolution glimpse of an RNA-catalyzed reaction thought to be fundamental to the origin of life.

P-48

Analysis of the structure and dynamics of a tetracycline-dependent aptamer by pulse-EPR spectroscopy

D. Wunnicke[1], E. Feresin[1], J. Holterhues[1], E. Bordignon[1], D. Strohbach[2], J. Weigand[4], S. Mueller[3], B. Suess[4], H.-J. Steinhoff[1]
[1]University Osnabrueck, Germany, [2]University Bochum, Germany, [3]University Greifswald, Germany, [4]University Erlangen-Nuernberg, Germany

Natural riboswitches are present in non-coding regions of mRNA and gene expression occurs in absence and switched off in presence of a specific ligand. *In vitro* selected synthetic RNA aptamers behave like natural riboswitches and recognize their ligands with high specificity and affinity. Structural analysis of aptamer-ligand complexes showed that complex binding is often accompanied by structural and dynamic changes of the aptamer.
A secondary structure model of a tetracycline-(tc-)dependent aptamer was suggested with two single stranded regions involved in tc binding. Continuous wave EPR measurements performed on spin labelled aptamers indicate Mg^{2+}-induced dynamic and polarity changes. The spin label mobility of selected positions in the aptamer and the inter-spin distances (<2nm) do not show changes upon tc-binding. This indicates that the aptamer in the absence of tc adopts a conformation very similar to that of the activated state.
To verify the assumption we apply pulsed EPR methods (DEER) to determine inter-spin distances in the 2-8 nm range. Our results reveal an inter-spin distance in the RNA double mutant U12/56 of 3.7 ± 0.2 nm in absence and 3.8 ± 0.2 nm in presence of tc which confirms the previous assumption.

Abstracts

– Lipid biophysics –

P-49

Actinoporin binding to lipids studied by isothermal titration calorimetry

J. Alegre-Cebollada, E. Herrero-Galán, Á. Martínez del Pozo, J. G. Gavilanes
Department of Biochemistry and Molecular Biology I, Faculty of Chemistry, Complutense University, Madrid, Spain

Actinoporins are a family of soluble proteins produced by sea anemones with the fascinating ability to interact with membranes, change their conformation, oligomerize and form a toxic pore. A lot of work has been done in order to elucidate their mechanism of pore formation, including X-ray crystallography, NMR, fluorescence and circular dichroism, hemolysis and leakage assays, conductivity measurements, FTIR, ultracentrifugation, mutagenesis, etc. However, there is still a scarce of experiments regarding the thermodynamics of the transition of actinoporins to the active pore. In this work, isothermal titration calorimetry (ITC) was employed to gain insight into the thermodynamics of pore formation by Sticholysins I and II (StnI and StnII), the actinoporins from the Caribbean Sea anemone *Stichodactyla helianthus*. Titration curves can be reasonably adjusted to a model of binding of the lipids to one site in the protein, in agreement with the putative model of pore formation. However, the stoichoimetry of the interaction cannot be interpreted taking into account the same rules that govern the interaction of soluble molecules. In this study, some interesting mutants of StnII were also analyzed and compared to the wild-type protein. The results obtained are discussed in terms of the current knowledge of the mechanism of pore formation, together with other results obtained from some of the techniques outlined above.

P-50

Interactions of proteins with lipid monolayer: structural studies by cryo-electron microscopy

A. Arduin[1], P. J. Gaffney[1], M. Katan[2], E. Morris[2]
[1]Imperial College London, London, UK, [2]The Institute of Cancer Research, London, UK

This study focuses on the function and regulatory mechanisms of phosphoinositide-specific phospholipase C isoforms (PLC), in particular on the newly identified PLCε, with the aim to gain more insight in that respect by structural studies using cryo-EM. The conditions to grown 2D crystals of PLCε on a lipid monolayer have been investigated to exploit advantages in terms of environment and preservation of the protein.
The PLC family comprises a diverse group of enzymes that catalyze the hydrolysis of phosphatidylinositol under the control of cell surface receptors and effectors which are related with cancer. These proteins have, to some extent, a common structural organization and a similar mechanism of function which is not yet understood in detail. The formation of 2D crystals on a lipid monolayer is being investigated, taking into account the properties of the lipid layer, the sample preparation and methods for linking PLC to the membrane with high affinity. This approach has been chosen because it allows PLCε analysis in an environment similar to the *in vivo* environment and because structural studies using cryo-electron microscopy of 2D crystal recently led to the successful investigation of proteins associated with lipid-membranes.
Large molecules and their complexes are more suitable for analysis using electron microscopy and this provides further reason to focus on PLCε which is the largest of all PLC isoforms. Furthermore, studies on PLCε can lead to further insights into all the other PLC isoforms.

P-51

Interaction of polyamines and CAMPs with negatively charged lipid membranes: a calorimetric study

A. Arouri[1], M. Dathe[2], A. Blume[1]
[1]Institute of Chemistry, MLU Halle-Wittenberg, Halle, Germany, [2]Research Institute of Molecular Pharmacology, Berlin-Buch, Germany

In spite of the large efforts undertaken to bring some of the cationic antimicrobial peptides (CAMPs) to the drug market, the lack of a thorough molecular-based understanding of their mechanisms of action impedes the progress in this field. Based on the fact that a net cationic charge and an amphipathic structure are essential for the functionality of CAMPs, the study of the interaction of polyamines with negatively charged lipid vesicles would help to elucidate the behavior of CAMPs by isolating the effects due to pure electrostatic interactions with lipid membranes. Polyamines, like spermine and spermidine for instance, are known to interact merely with negatively charged lipid vesicles by means of electrostatic attraction and hydrogen bonding (no hydrophobic interaction). Unlike CAMPs, these polyamines by themselves can neither penetrate or fuse lipid vesicles nor disturb the hydrophobic core of the lipid membrane. We used microcalorimetry (DSC and ITC) to study the interaction of polyamines with negatively charged lipid vesicles. The results obtained with the polyamines provided the background to resolve our results obtained with the following CAMPs: a) the lysine-containing KLAL peptides (+6); b) the arginine-containing C-RW (cyclic) and Ac-RW (acyclic) peptides (+4). Further experiments are being carried out to explore the influence of the polyamine net charge, charge distribution, geometry, spacer type and spacer length.

P-52

Lipid membrane based biosensor using localized surface plasmon resonance spectroscopy

C. L. Baciu, S. Pierrat, J. Becker, C. Soennichsen, A. Janshoff
Institute for Physical Chemistry, Welder Weg 11, Johannes Gutenberg University, 55128 Mainz, Germany

Multi-component lipid bilayers determine the basic structure of biological membranes. Their properties and interactions with different ions and molecules are essential for the well controlled interplay, material exchange and signaling between compartments performing different functions. Here we propose a nanoscale optical biosensor that employs the refractive index sensing properties of subwavelength nobel-metal structures for monitoring the state of the lipid bilayer under the influence of different agents as well as for characterization of specific and/or nonspecific interactions of the lipid membrane with different biomolecules. The biosensing chip consists of structured lipid membranes covering the sensing nanoparticles (e.g. gold nanorods) that are electrostatically immobilized on functionalized glass substrate. The change in the local refractive index is detected as a change in the localized surface plasmon resonance (LSPR) wavelength. The strong spatial localization of the sensing events allows for detection of very low levels of analyte and the possibility for array bioassays, with high density of sensing elements.

Abstracts

– Lipid biophysics –

P-53

Modulating lipid membrane properties using membrane proteins

R. Batchelor, M. Lorch
Department of Chemisty, University of Hull, Hull, UK

The activities of integral membrane proteins are known to be affected by the physical properties of the surrounding lipid bilayer. With the aim of modulating protein structure
an integral membrane protein enzyme have be used to alter the characteristics of
a lipid membrane.
The effect of the enzyme action on the lipid bilayer have been characterised using a variety of solid state NMR techniques. Lipid order parameters have been determined by measuring 13C-1H dipolar couplings. And static 31P and 14N experiments have produced
further information on the dynamics of the bilayer.
For the first time we have shown how a membrane protein involved in lipid metabolism changes the physical properties of the membrane in which it resides.

P-54

Interactions of fluoroquinolone with phospholipids: modification of their physicochemical properties

H. Bensikaddour[1], E. Goormaghtigh[2], M.-P. Mingeot-Leclercq[1]
[1]Université catholique de Louvain, FACM, Bruxelles, Belgium, [2]Université Libre de Bruxelles, SFMB, Bruxelles, Belgium

Probing drug/lipid interactions at the molecular level represents an important challenge in pharmaceutical research and membrane biophysics.Novel insights on these interactions are reported in this study by using steady-state anisotropy fluorescence and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR).Two fluoroquinolones have been compared in this study, ciprofloxacin (CIP) and moxifloxacine (MXF).The selected phospholipids were DPPG, DPPC or phospholipids mixtures (DOPC:DPPC),and (DOPC:DPPG).Our results showed that all lipids vesicles tested bound to the CIP antibiotic with a stoichiometry of one (1:1).The binding constants K_{app} were in the order of $10^5 M^{-1}$ and the affinity appeared dependent on the negative charge. CIP bound to lipids vesicles with the following preference: DPPG > DOPC: DPPG (1:1) > DOPC: DPPC (1:1).No variation of anisotropy signal was detected in presence of MXF. ATR-FTIR experiments showed that CIP decreased the surface of the wagging CH_2 of DPPC lipid, in contrast, MXF increased it by two folds. Furthermore, the frequencies of CH_2 stretching vibrations of DPPC as a function of temperature indicated that melting temperature decreases only in the presence of CIP.These data demonstrated that the interactions of CIP and MXF with lipids are different since CIP increases DPPC disorder and decreases the lipid melting temperature, while MXF aligns phospholipids sn_2 chains of DPPC.Finally, this work highlights the biophysical applications of ATR-FTIR and anisotropy fluorescence to investigate lipids-drug interactions.

O-55

Membrane deformations induced by proteins

L. Berland[1], W. Römer[1], J. Solon[1], Y. Gaudin[2], P. Sens[3], L. Johannes[1], P. Bassereau[1]
[1]Institut Curie, Paris, France, [2]Unité de Virologie Moléculaire et Structurale, Gif-sur-Yvette, France, [3]Ecole Supérieure de Physique et Chimie Industrielle, Paris, France

Endocytosis, exocytosis, membrane traffic between organelles, virus/toxin entry, or budding from cell imply membrane deformations. The mechanisms for membrane deformations by proteins are currently actively investigated. Giant vesicles (GUV) are interesting model membrane systems because they are composed of a very limited number of components compared to cellular membranes, and their membrane tension can be tuned. The deformations induced by the interaction with a specific protein or any other additional components to the system, can then be directly monitored and the deformation mechanism eventually understood. Viral and toxin proteins are able to induce membrane invaginations in GUVs in absence of other cellular machinery. We will show examples with the matrix protein of the VSV virus interacting with negatively-charged vesicles, with the B subunit of the Shiga toxin or the Cholera toxin interacting with GUV containing their lipid receptor, Gb3 or GM1 respectively. Possible mechanisms for membrane deformations will be presented.
This type of in vitro study should allow us to eventually better understand membrane cellular transport.

P-56

Assaying BAR domain curvature affinity

V. K. Bhatia[1], K. Madsen[2], P.-Y. Bolinger[1], I. Makarov[1], H. T. McMahon[3], U. Gether[2], D. Stamou[1]
[1]Bionanotechnology lab, Nano-Science Center, University of Copenhagen, Denmark, [2]Molecular Neuropharmacology lab, University of Copenhagen, Denmark, [3]MRC, Laboratory of Molecular Biology, University of Cambridge, UK

BAR (Bin, Amphiphysin, Rvs) domain proteins are involved in a large number of cellular membrane reshaping processes such as endo- and exocytosis of synaptic vesicles[1]. BAR domain proteins are crescent shaped dimers which can sense curvature and are proposed to bind on negatively charged membranes with different affinities depending on curvature[2]. So far this binding behaviour has been addressed in a complex bulk assay with centrifugation and SDS-PAGE. We present a high-throughput single lipid vesicle assay[3] to test the curvature dependant binding properties of BAR proteins. Downscaling the assay to single nanometer sized vesicles eliminates severe ensemble averaging issues, consumes 1/1000th of the material and speeds up the assay dramatically. Fluorescence intensities of immobilized reconstituted brain lipid vesicles allowed us to measure accurately their size/curvature and the respective densities of BAR proteins. The assay allowed us for the first time to calculate BAR-membrane binding affinity and on/off-rates for Endophilin, a BAR domain containing protein.

1. McMahon HT & Gallop JL. *Nature* **438**, 590-596 (2005).
2. Peter BJ *et al*. *Science* **303**, 495-499 (2004).
3. Stamou D, Duschl C, Delamarche E & Vogel H. *Angewandte Chemie-International Edition* **42**, 5580-5583 (2003).

Abstracts

– Lipid biophysics –

P-57

Membrane modification by novel N-oxides

K. Bielecki[1], H. Kleszczyńska[2], J. Sarapuk[2], Z. Trela[2], D. Bonarska-Kujawa[2], A. Dziamska[2]
[1]Department of Plant Nutrition, University of Environment and Life Sciences, Wrocław, Poland, [2]Department of Physics and Biophysics, University of Environment and Life Sciences, Wrocław, Poland

New single alkyl chain N-oxides (NTA), differing in length of the alkyl chain, were synthesized for use as biologically active compounds. Potential activity was checked in a series of experiments that permitted to determine their membrane and antioxidative efficiencies.
Model (BLM) and biological membranes (pig erythrocytes and algae) were used to study the membrane activity of NTA, the measure of the activity being efficiency to break BLM membranes, hemolyse erythrocytes, and change alga membrane resting potential. Also, NTA influence on the growth of cucumber, chlorophyll content and potassium leakage from cucumber was studied.
The cucumber experiments showed that the ability to change the parameters studied increased with NTA alkyl chain length while the hemolytic and BLM experiments showed also that there existed optimal length of the alkyl chain at which the interaction between NTA and used membranes was greatest.
The antioxidative properties determined from studies on protection of ghost membranes against UV induced peroxidation - where NTA were incorporated into membranes at concentrations far below hemolytic ones – were was quite good.
The work was sponsored by the Polish Research Committee (KBN), grant no. 2 P04G 088 27.

P-58

From Anomalous Swelling to Main Transition: the DC13PC Bilayers.

P. Brocca, L. Cantù, E. del Favero, M. Corti, S. Motta, C. Nodari
Dept. of Medical Chemistry, Biochemistry and Biotechnologies, L.I.T.A., V.le F.lli Cervi 93, 20090 Segrate, Italy.

The experimental description of the lipid main transition in terms of a first order broadened transition is long lived, independently from the observation of the anomalous swelling behavior. Few experimental evidences, as that of the Lx phase of $DC_{12}PC$ membranes, and molecular dynamics studies have indicated more complex transition regime. We focus here on the structural evolution of $DC_{13}PC$ membranes along the temperature region approaching and across the chain-order transition from the L_α to P_β' phase, along a cooling path, as followed by small-angle and wide-angle x-ray scattering. Snapshots of a tenth of a second at fixed temperature, in the absence of the dragging effect of a continuous scan, were collected. Results support the "*evolving membrane*" concept of molecular-dynamics-simulation, here in a bulk, not-supported membrane system. In particular, across the transition a coexistence of two phases occurs: a fluid phase keeping on swelling to longer distances until completely voided, and a raising P_β' gel phase with a constant interlamellar distance.. The analysis is completed by calorimetric and densitometric measurements showing anomalies in the specific heat along both the swelling and the transition regions that is associated to a two-step process. Analogous approach has been applied to study the transition of the same lipid organized in large unilamellar vesicles. Waxs, calorimetric and densitometric measurements show the existence of a double step main transition also in this single component nanosized closed bilayer.

P-59

A new membrane destabilization mechanism: electrostatic repulsion promoted by lipopeptide Surfactin

S. Buchoux, M. Garnier, E. J. Dufourc
UMR 5248 CNRS - Université Bordeaux 1, Pessac, France

Surfactin (SF) is a lipopeptide composed of 7-amino-acid-long peptide ring (ELLVDLL) completed by an aliphatic chain (12 C atom long). Due to the presence of both the hydrophobic chain and the hydrophilic ring (2 amino acids are anionic), SF exhibits interesting physical and is known to have critical effects on biomembranes, leading to biological properties such as: hemolysis, antimycoplasmal and antimicrobial activities or destabilization of enveloped viruses (HSV and HIV-1). Regarding the morphology of SF, the way it interacts with membranes is unclear and can hardly be compared with known mechanisms of membrane destabilization induced by helical amphipathic peptides (barrel and carpet mechanisms). Our approach is to use NMR (solid state and HR-MAS) to study SF in interaction with model membranes (neutral and negatively charged) in order to understand the mode of action. Our ^2H NMR results show that SF has a slight effect on DMPC (zwitterionic) liposomes (μm scale) but induces a complete destabilization of negatively charged membranes at pH 7 (DMPG/SM, DMPC/DMPG and DMPS/DMPC) leading to formation of smaller objects (nm scale, confirmed by light scattering experiments). At pH 4.5, where the lipid membrane becomes zwitterionic (SF being still anionic) an effect similar that found with DMPC is observed. This leads to the proposal of a new membrane lysis model based on electrostatic repulsion and membrane curvature changes. HR-MAS studies and MD experiments are currently performed to obtain SF structure in lipid bilayers and will lead to the proposal of a model at atomic resolution.

P-60

Catalytic digestion of phosphodiester bonds by cationic amphiphilic drugs – a structural approach

D. R. Casey[1], S. C. Sebai[1], G. C. Shearman[1], O. Ces[1], R. V. Law[1], A. D. Gee[2], R. H. Templer[1]
[1]Dept. of Chemistry, Imperial College, London, U.K., [2]GSK Clinical Imaging Centre, London, U.K.

In a previous paper, (Baciu *et al*, *Phil. Trans. Soc. A.,* 2006, **364**, (1847), 2597-2614), we reported for the first time direct evidence of a new mechanism of transport of cationic amphiphilic drugs (CADs) across model cell membranes of dioleoylphosphatidylcholine (DOPC). The mechanism of this transport was shown to be due to the hydrolysis of the ester moieties within the lipids, rapidly forming drug-containing micelles of lyso-lipids. It was proposed that this reaction was catalysed by the acidic proton of the CAD in its charged state.
Present studies explore the effects of CAD structure upon the rate of this effect, by varying the steric accessibility and basicity of a drug-like molecule's cationic centre. These studies are undertaken via fluorescence microscopy, HPLC and NMR measurements, with a view to developing a quantitative relationship between a compound's physico-chemical properties and the rate at which it catalyses membrane degradation.

Abstracts

– Lipid biophysics –

P-61

The physico-chemical properties of phosphoinositol lipids drive changes in membrane curvature

O. Ces[1], R. H. Templer[1], X. Mulet[1], R. Woscholski[2]

[1]Chemical Biology Centre, Imperial College, London, Exhibition Road, London SW7 2AZ, U.K., [2]Division of Cell and Molecular Biology, Imperial College, London, Exhibition Road, London SW7 2AZ, U.K.

Current models for trafficking pathways include mechanisms where membrane curvature sensing and generating proteins stabilize curved membrane interfaces, often in response to locally elevated levels of phosphoinositol lipids. Given that phosphoinositol lipids play such a key role in signal transduction processes it seems surprising that studies of the phase behaviour of this class of lipids, in particular with respect to their curvature inducing properties have been limited. We show for the first time, that the physico-chemical properties of this class of lipids are in themselves capable of driving dramatic changes in membrane curvature, exceeding the ability of classically recognised non-bilayer lipids such as phosphatidylethanolamine to promote curved mesophases.

P-62

The effect of sterols on the formation of liquid ordered phase in model membrane

L. Chen[1], J. Lawrence[2], Z. Yu[3], P. Quinn[1]

[1]Department of Biochemistry, King´s College London, U.K., [2]Department of Pharmacy, King´s College London, U.K., [3]Department of Chemistry, Tsinghua University, Beijing, China

Lipid rafts are considered to exist in a liquid-ordered (L_o) state characterized by tight ordering but relatively high lateral mobility. There is convincing evidence to suggest that ordered lipid domains composed of cholesterol and saturated lipids play an important role in cellular processes. The other sterols with widespread occurrence in plants and microorganisms differ structurally from cholesterol only with regard to their side chain. Their ability to form ordered phases is of interest. We compared the effects of cholesterol, stigmasterol, desmosterol and ergosterol on the thermotropic phase behaviour of phospholipid bilayers using differential scanning calorimetry and X-ray diffraction methods. Aqueous codispersions of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) with sterols in a molar ratio of 2:1 were examined as this stoichiometry is considered to form L_o phases. Calorimetric experiments demonstrated that all sterols decreased the temperature of the main phase transition of DPPC at 41.2^oC. Wide-angle X-ray diffraction studies showed that cholesterol and desmosterol have a similar effect on the chain packing such that wide-angle d-spacings increase from 0.45 nm to 0.47 nm upon heating from 30^oC to 60^oC. This change is believed to be a characteristic of L_o phase. Codispersions of DPPC with stigmasterol and ergosterol, by contrast, showed a progressive increase in wide-angel d-spacings from 0.43 nm to 0.46 nm during heating over the same temperature range consistent with a transition from gel to liquid crystal or L_o phase.

P-63

Temperature dependence of Apparent molal volume, Viscosities and B-coefficients of Carbohydrate monomer in aqueous & in aqueous Geraniol oil solution at 298.15, 304.15 & 310.15.

N. Choudhary, M. Singh

Research Lab, Deshbandhu College, University of Delhi, 110019 New Delhi, India

Density $\rho/10^3 kgm^{-3}$, viscosity $\eta/10^{-1} kgm^{-1}s^{-1}$ and surface tension γ/Nm^{-1} at three different temperatures 298.15, 304.15 and 310.15 K for the solutions of carbohydrate monomers viz. D-glucose, D-fructose and D-mannitol at different concentrations have been determined in water. The same parameters of the aqueous solution of geraniol oil were also determined at different concentrations. For the ternary systems aqueous geraniol solution was used as the solvent for making the solutions of carbohydrate monomers. The ρ values were used for apparent molar volume ($V\phi/10^{-6} m^3 mol^{-1}$), η and γ calculations; η fitted in extended Jones-Dole equation for B/kg $mol^{-1}$and D/ (kg $mol^{-1})^2$ coefficients. The γ and $V\phi$ were regressed against molality m, for limiting surface tension $\gamma^\circ$and apparent molal volume $V^\circ\phi$ at $m \rightarrow 0$. Partial molal volume $V'^\circ\phi$ of water calculated from ρ values. The data have been evaluated using Jones-Dole equation and the obtained parameters have been interpreted in terms of solute-solvent interactions. The activation parameters of viscous flow have been obtained which depicts the mechanism of viscous flow. The limiting apparent molal volume and B-coefficient value were splits in the contribution of polar and nonpolar groups. These data are rationalized on the basis of hydrophilic and hydrophobic interactions between the groups present in the systems taken.

P-64

Using UV to shed light on membrane stress

E. H. Clay, R. H. Templer, D. Davies, O. Ces, P. J. Gaffney

Imperial College London, UK

The role of curvature and membrane stress as key driving forces in biological systems are still being determined. Small changes in membrane composition have already been shown to affect the folding and function of membrane proteins. However quantification of the effects has been difficult. In this territory new tools are needed to investigate, in detail, the spacio-temporal changes within the membrane.

We present a novel and flexible synthetic route towards a range of dipyrenyleacyl lipids (ex. 344nm, S_2 375nm, S_1 390nm, E 450nm). Applications of these dipyrenyl lipid probes for studying the stored curvature elastic stress in model membranes (including membrane depth dependencies) and in *in vivo* systems are discussed. The dipyrenyl lipids have shown increases in pressure as the amount of 1,2-di-oleoyl-sn-glycero-3-phosphatidylethanolamine is increased in 1,2-di-oleoyl-sn-glycero-3-phosphocholine systems while the addition of 1,2-di-oleoyl-sn-glycerol reduces the pressure in 1,2-di-oleoyl-sn-glycero-3-phosphatidylcholine. The addition of simple proteins has been carried out and revealed interesting phenomena in the model membrane systems.

Abstracts

– Lipid biophysics –

P-65

Intermonolayer friction and shear viscosity of lipid membranes by molecular dynamics simulations

W. K. Den Otter, S. A. Shkulipa, W. J. Briels
Computational Biophysics, University of Twente, Enschede, The Netherlands

The flow behaviour of lipid bilayer membranes is characterized by a surface shear viscosity for in-plane shear deformations and an intermonolayer friction coefficient for slip between the two leaflets of the bilayer. Experimental measurements of these properties are delicate and rely on theoretical interpretations which are still under debate. We present molecular dynamics simulations of lipid membranes exposed to perpendicular and parallel shear flows, which provide direct access to the shear viscosity and the intermonolayer friction, respectively. For lipids with two identical tails, the surface shear viscosity rises rapidly with tail length, while the intermonolayer friction coefficient is less sensitive to the tail length. Interdigitation of lipid tails across the bilayer midsurface, as observed for lipids with two distinct tails, strongly enhances the intermonolayer friction coefficient, but hardly affects the surface shear viscosity. Having established the flow properties from non-equilibrium simulations, we are now in a convenient position to validate the available theories coupling flow properties to equilibrium dynamics: the Saffman theory relating tracer-diffusion to the shear viscosity, and the Seifert-Langer theory relating undulatory dynamics to intermonolayer friction. The simulation results are also compared against the available experimental data.
http://cbp.tnw.utwente.nl/Research/amphi.html

P-66

Exploration of biophysical properties and potential functions of cubic cell membranes

Y. Deng, F. Margadant, M. R. Wenk
Departments of Physiology and Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Membranes are of fundamental importance for biological systems. They provide for cellular compartmentalization and control of the internal cell environment. The biophysical properties of the membrane lipids and proteins play a key role in determining membrane morphology. Far from being a simple flat sheet, cell membrane can fold itself into 3D nano-periodic cubic structures. The same cubic geometry is well studied in other disciplines such as mathematics, physics and polymer chemistry. Although cubic membranes have been observed in numerous cell types and under various stress conditions, knowledge about their biophysical properties and potential function is scarce. Progress in understanding the role of membrane lipids on cubic membranes formation is achieved through studying lipid profile and liposomal construction of lipids extracted from cubic membranes in our laboratory. We have also investigated the physical properties of cubic membranes in two representative models of mitochondria with cubic membrane organization: (1) photoreceptor cells in the retina of small mammals (Tree Shrew), (2) amoeba *Chaos carolinense.* Our simulation of the optical properties of cubic membranes in photoreceptors revealed that they might act similar to a material of an average elevated refractive index but with astoundingly low interference filter properties. Furthermore, our recent experimental data show that isolated amoeba mitochondria with cubic membrane structure are able to uptake and retain macromolecules such as DNA, suggesting a potential role of cubic membranes in intracellular transportation.

P-67

Lateral segregation of membrane constituents driven by the membrane bending energy

J. Derganc
Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Slovenia

Freely mobile membrane constituents (e.g., lipids, proteins, or membrane domains) can migrate to the membrane regions of their preferred curvatures. This process of lateral segregation decreases the elastic energy of the membrane but at the same time decreases the system's entropy, and thus the resulting equilibrium membrane composition is a balance between these two effects. In this work the standard Helfrich model of membrane elasticity is applied to inhomogeneous membranes in order to estimate the extent of lateral segregation in the membrane of Golgi cisternae. Within this model the membrane spontaneous curvature is not a constant all over the membrane but depends on the local membrane composition – it is calculated as a weighted average of the spontaneous curvatures of the membrane constituents, using the local concentrations of those constituents as the weighting factors. The Golgi cisterna was modeled as a discoid with only two different curvatures – the flat cisternal membrane and the highly curved membrane in the cisternal rims. For simplicity, the membrane of only two different constituents was considered. The analysis provides a simple analytical result for estimating the extent of lateral segregation, without any non-measurable parameters.

P-68

A spin label study of the interaction of the anthraquinone barbaloin with lipid bilayers

E. L. Duarte[1], T. R. Oliveira[1], D. S. Alves[2], V. Micol[2], M. T. Lamy[1]
[1]Instituto de Física, Universidade de São Paulo, São Paulo, Brazil, [2]Instituto de Biologia Molecular y Celular, Universidad Miguel Hernández, Alicante, Spain

Barbaloin, a commercial plant extract, is used in cosmetics, food and drugs. It has been shown that barbaloin interacts with DMPG (dimyristoyl phosphatidyl glycerol) model membranes, altering the bilayer structure (Alves, Pérez-Fons, Estepa, Micol, Biochem. Pharm. 2004, 68, 549). Considering that EPR (electron paramagnetic resonance) of spin labels is one of the best techniques to monitor structural properties at the molecular level, the alterations caused by the anthraquinone barbaloin on phospholipid bilayers will be discussed here via the EPR signal of phospholipid spin probes intercalated into the membranes. In DMPG at high ionic strength (10 mM hepes pH 7.4 + 100 mM NaCl), a system that presents a gel-fluid transition around 22 ^{o}C, 20 mol % barbaloin turns the gel phase more rigid, does not alter much the fluid phase packing, but makes the thermal transition less sharp. However, in low salt DMPG dispersion (10 mM hepes + 2 mM NaCl), which presents a rather complex gel-fluid thermal transition (Lamy-Freund, Riske, Chem. Phys. Lipids, 2003, 122, 19), barbaloin strongly affects the bilayer properties, both in the gel and fluid phases. The position of barbaloin in DMPG bilayers will be discussed based on the EPR results, in parallel with data from sample viscosity, DSC (differential scanning calorimetry), and SAXS (small angle X-ray scattering).
Financial support: USP, FAPESP, CNPq

Abstracts

– Lipid biophysics –

P-69

Protective effect of flavonoids from Aronia melanocarpa on hypercholesterolemia in human erythrocyte

P. Duchnowicz[1], A. Staron[1], M. Broncel[2], A. Ziobro[1], M. Koter-Michalak[1]

[1]Department of Environment Pollution Biophysics, University of Lodz, 12/16 Banacha St., 90–237, Lodz, Poland, [2]Department of Clinical Pharmacology, Medical University of Lodz, 1/3 Kniaziewicza St., 91–347, Lodz, Poland

Hyperlipidemia is one of major risk factors of atherosclerosis. Cholesterol affects the normal rheology of blood through its interaction with erythrocytes. Flavonoids have antioxidant and anticancer activities. We determined the effect of *Aronia melanocarpa* flavonoids on the hypercholesterolemic erythrocytes both *in vivo* and *in vitro* study. The study involved 25 patients with hyperlipidemia and 10 healthy donors. The patients were selected on the basis of the plasma cholesterol higher than 200 mg/dL and LDL-cholesterol higher than 150 mg/dL. Patients were on diet supplemented with *Aronia melanocarpa* extract for 2 months. Blood was tested at the beginning and after each month. Erythrocytes from patients and healthy donors were incubated with *Aronia melanocarpa* extract for 24 hours at 37°C. *In vivo* study. Decreased of cholesterol concentration and lipid peroxidation after 2 months was observed. No changes in activity of ATPase was observed. Increase in superoxide dismutase and peroxidase activity, and decrease in catalase activity were observed after 2 months. *In vitro* study. No significant reduction of cholesterol levels and no changes in ATPase activity was observed in control group. Decrease in lipid peroxidation after 24 h incubation was observed for controls. Incubation of patients' erythrocytes with the extract decreased cholesterol concentration and lipid peroxidation and increased ATPase activity.

P-70

"Juicy membranes"- the interaction of limonene and it´s oxidation product on DMPC membranes

L. Duelund, A. Amiot, A. Fillon, O. G. Mouritsen

MEMPHYS-Center for Biomembrane Physics, Department of Physics and Chemistry, University of Southern Denmark, DK 5230 Odense M, Denmark

The monocyclic monoterpene limonene (4-isopropenyl-1-methyl-1-cyclohexene) is among the most common secondary metabolites in plants, being produced by more than 300 different plant species. A very rich and well known source of limonene is the rinds of oranges. The oil obtained from the rind contains up to 95% limonene, and is used for many different purposes. It is used as a food additive, a fragrance in household cleaning products, and as an industrial solvent, often replacing mineral oil.

Despite its presence in many plants and its widespread industrial use no detailed studies of its influence on (model) membranes have been published so far. For that reason, we have started the investigation by first determining the membrane - buffer partition coefficient (K) by isothermal titration calorimetry (ITC). Further, we have looked at how the phase transition of the membrane is affected by limonene, using differential scanning calorimetry (DSC). Finally, we have examined how limonene affects the fluidity of the membrane, using electron spin resonance spectroscopy (EPR).

Using the same techniques, we have studied the influence of three major oxidation products of limonene. These are: Perillyl alcohol (4-isopropenyl-1-cyclo-hexenyl-methanol), perillaaldehyde (4-isopropenyl-1-cyclo-hexene-1-carbox-aldehyde), and finally perillic acid (4-isopropenyl-1-cyclo-hexene-1-carboxylic acid), in both its protonated and deprotonated forms.

P-71

Role of the N-terminal amphipathic helix of a N-BAR domain in membrane tubulation

F. Fernandes[1], L. M. S. Loura[2], A. P. A. Matos[3], F. Alexander[1], M. Prieto[1]

[1]Centro de Química-Física Molecular, Instituto Superior Técnico, Lisboa, Portugal., [2]Centro de Química and Departamento de Química, Universidade de Évora, Évora, Portugal, [3]Departamento Biomateriais, FMDUL, Hospital Curry Cabral, Lisboa, Portugal.

A large group of proteins are recognized to have membrane remodeling properties, such as the ability to induce tubulation of liposomes. The mechanism by which this effect is exerted has been proposed to be the burying of amphipathic helices into the lipid bilayer. For proteins presenting BAR domains, removal of a N-terminal amphipathic alpha-helix results in much lower membrane tubulation efficiency. Here we studied the interaction of a peptide corresponding to this segment (H0-NBAR) with model lipid membranes. The peptide bound avidly to anionic liposomes but partitionate weakly to zwitterionic bilayers. Binding to anionic lipids is insensitive to both lipid structure and membrane curvature. Interestingly, it is shown that after membrane incorporation the peptide oligomerizes as an antiparallel dimer. Through monitoring the effect of H0-NBAR on liposome shape by electron microscopy, and on the fluorescence anisotropy of different membrane probes, it is clear that membrane morphology is not radically changed, but lipid packing is increased. We conclude that H0-NBAR alone is not able to induce vesicle curvature, and its function must be related to the mediation of oligomerization of BAR domains, or to the presentation of the BAR domain to the membrane with the correct orientation.

P-72

Contribution of charged and neutral lipids to membrane elasticity and adsorption of polypeptides

O. A. Finogenova, D. V. Filinsky, Y. A. Ermakov

Frumkin Institute of physical chemistry and electrochemistry RAS, Moscow, Russia

The electrostatic phenomena due to the presence of a charged lipid component in the membrane are commonly assumed to be the principal factor in their interaction with small and macromolecular substances in biological cells. To model these phenomena, we used the artificial planar bilayer lipid membranes (BLM) and liposomes consisting of charged (phosphatidylserine, PS, and cardiolipin, CL) and neutral (phosphatidylcholine, PC) components with different ratios. The boundary potential was registered at BLM by Intramembranous Field Compensation technique; the surface potential was found from multilayer liposome electrophoretic mobility; and membrane bending elasticity was evaluated by micropipette technique applied to giant vesicles. It was found that 1) bending rigidity increased with PS/PC ratio up to 15 mol % and the effect was about 10 times more pronounced than predicted by the simplest theory; 2) the adsorption of lysine based polypeptides was partially irreversible and induced different changes in membrane boundary and surface potentials, which were not proportional to the charge density of CL/PC membranes. The membrane elasticity was described qualitatively if the immersion depth of an "incompressible" charged surface into the bilayer changed linearly with the PS/PC ratio. To explain the discrepancy between the two data sets and the simplest electrostatic models at the molecular level, changes in lipid packing and/or in polar heads hydration are suggested.

Abstracts

– Lipid biophysics –

P-73

Membrane activity of sulphonic derivative of quercetin and its mixture with organotin in vitro

J. Gabrielska, J. Miśkiewicz, J. Kupiec, S. Przestalski
Wroclaw University of Environmental and Life Sciences, Dept. Physics & Biophysics, 50-375 Wroclaw, Poland

Quercetin (Que) is a flavonoid that shows high antioxidative properties *in vitro*. It is hardly soluble in water. We have investigated the antioxidative activity of its water soluble analog, sulphonic acid of quercetin (QSA), compared to that of Que. Studies on phosphatidylcholine liposome and LDL membranes, using the TBARS test, showed that QSA exhibits in all the cases (oxidation by UV, Fe/ascorbate and AAPH) and in the 0.3 - 20 μM range a lower antioxidative activity than Que. Also the antioxidative abilities of QSA in the presence of phenyltin chlorides, i.e. organometallic compounds that induce lipid peroxidation in the presence of UV, were lower than the antioxidative abilities of Que. As confirmed by our studies, the probable reason for weaker QSA activity, compared to Que, is its lower ability to scavenge free radicals of diphenylpicrylhydrazil, a greater susceptibility to photodestruction and oxidation in the presence of AAPH. QSA induces a leakage of carboxyfluorescein from liposomes PC to a lesser degree than Que does, which suggests that its lower adsorption on the membranes. However, the lowered chelating properties of QSA in the presence of liposome towards phenyltin as compared with Que (constants of complex formation: $K_{Que:DPhT} > K_{Que:TPhT} \cong K_{QSA:DPhT} >> K_{QSA:TPhT}$) and smaller degree of membrane stiffness caused by QSA, found fluorimetrically, may be the explanation for the weaker protection of membranes against phenyltins induced peroxidation, compared with action of Que. Work was sponsored by the Polish State Committee for Sci. Res., grant no 2 P04 G 089 27.

P-74

Cholesterol-driven lamellar phases

D. L. Gater, R. V. Law, J. M. Seddon
Department of Chemistry, Imperial College, London

The role of cholesterol (Chol) in the formation of lateral heterogeneities (or 'lipid rafts') in biological systems remains a question of great interest. In model membranes composed of dipalmitoylphosphatidylcholine (DPPC), which forms a fluid lamellar (L_α) phase above 41^oC, the addition of Chol has been found to lead to the formation of a 'liquid ordered' (L_o) phase in which the lipid acyl chains have predominantly all-*trans* conformations. Other studies have shown that the L_o phase is formed in mixtures of Chol with saturated chain lipids which themselves form a L_α phase when mixed with water, but are there any other amphiphilic systems where Chol is able to induce the formation of the L_o phase
Chol has been shown to induce the formation of a L_o phase when mixed with myristic, palmitic or stearic acids or with the single chained lipid, lyso-PPC. None of these lipids will form a lamellar phase when fully hydrated in the absence of Chol. We have extended our studies of mixtures of Chol and non-lamellar forming lipids to look at dipalmitoylglycerol (DPG), which is a secondary messenger in some cell signalling processes. DPG is too weakly amphiphilic to form lyotropic liquid-crystalline phases on its own in water, but is soluble in phospholipid membranes, where it tunes the interfacial curvature towards negative values, promoting the formation of inverse non-lamellar phases. The phase transitions of mixtures of DPG and Chol were identified using differential scanning calorimetry. Each phase was then characterised using a combination of small and wide angle x-ray scattering, and solid-state NMR.

P-75

1-Alkanols and membranes: A story of attraction

B. Griepernau[1], S. Leis[1], M. F. Schneider[2], M. Sikor[2], D. M. Steppich[2], R. A. Böckmann[1]
[1]Theoretical and Computational Membrane Biology, Center for Bioinformatics, Saarland University, Saarbrücken, Germany, [2]Department of Physics, Augsburg University, Augsburg, Germany

Although 1-alkanols have long been known to act as penetration enhancers and anesthetics, the mode of operation is not yet understood. In this study, long-time molecular dynamics simulations have been performed to investigate the effect of 1-alkanols of various carbon chain lengths onto the structure and dynamics of dimyristoylphosphatidylcholine bilayers. The simulations were complemented by microcalorimetry, continuous bleaching and filmbalance experiments. In the simulations, all investigated 1-alkanols assembled inside the lipid bilayer within tens of nanoseconds. Their hydroxyl groups bound preferentially to the lipid carbonyl group and the hydrocarbon chains stretched into the hydrophobic core of the bilayer. Both, molecular dynamics simulations and experiments showed that all 1-alkanols drastically affected the bilayer properties. Insertion of long-chain 1-alkanols decreased the area per lipid while increasing the thickness of the bilayer and the order of the lipids. The bilayer elasticity was reduced and the diffusive motion of the lipids within the bilayer plane was suppressed. On the other hand, integration of ethanol into the bilayer enlarged the area per lipid. The bilayer became softer and lipid diffusion was enhanced.

P-76

Advanced red blood cell thermal fluctuation analysis: a new method to quantify membrane elasticity

J. P. Hale[1], G. Marcelli[2], K. H. Parker[3], C. P. Winlove[1], P. G. Petrov[1]
[1]School of Physics, University of Exeter, Exeter, U.K., [2]Department of Mathematics, Imperial College of Science, Technology and Medicine, London, U.K., [3]Department of Bioengineering, Imperial College of Science, Technology and Medicine, London, U.K.

We propose a new experimental technique for red blood cell thermal fluctuation analysis, which makes it possible to quantify the cell membrane elastic moduli. The method is based on the comparison of the mean-square fluctuations in the shape of cell equatorial contours as observed using phase contrast microscopy to the equivalent fluctuation spectrum acquired in a coarse grained molecular dynamics simulation. The simulation is based on a network of virtual particles which interact via a harmonic potential and a dihedral angle potential and are subject to a constant volume and area constraints, which gives rise to finite values for the membrane bending and shear elastic moduli. Using this method, the elastic properties of individual red blood cells can be measured and their changes can be easily monitored in response to changing environmental conditions (temperature, solute concentration, osmotic pressure changes). We also present evidence of increased bending rigidity in red blood cells from diabetic individuals.

Abstracts

– Lipid biophysics –

P-77

Lipid membranes under the action of antimicrobial peptides (melittin and alamethicin).

M. Hanulová[1], S. S. Funari[1], J. Howe[2], K. Brandenburg[2]
[1]Hasylab at Desy, Hamburg, Germany, [2]Laborgruppe Biophysik, FZ Borstel, Borstel, Germany

Melittin (from bee venom) and alamethicin (from fungus Trichoderma viridae) are amphipathic α-helical antibacterial peptides. Both peptides cause lysis of model and bacterial membranes. We studied the impact of the peptides on the structure of model membranes by Xray diffraction and infrared spectroscopy in the temperature range 5 to 85^{o}C and lipid/peptide ratios 10 to 1000. The membranes were formed by phospholipids (POPC or POPE) or lipopolysaccharides (LPS) from *Salmonella minnesota*.
The interaction is facilitated by the amphipathicity of both peptides and membranes and the final structure is determined by the geometry of the molecules. So, the peptides insert into the POPE membrane (between the headgroups) while they only adsorb onto the POPC membrane. This reflects in the membrane structures. In POPE, peptide binding causes bilayer thinning in the lamellar phase as a result of pushing apart of the lipid headgroups. Cubic phases form at high temperatures which can perfectly accommodate both a peptide-filled and a peptide-free membrane as they contain parts with positive and negative curvature (headgroup-to-chain cross sections ratio bigger and smaller than 1, respectively). In POPC, peptide binding causes bilayer swelling, a result of increased distance between adjacent bilayers due to peptide adsorption.
Melittin also condensed lipopolysaccharides into ordered structures. This is important in the prevention of the septic shock that is induced by the release of bacterial LPS into the organism after the bacteria are killed by an antibiotic.

P-78

Antimicrobial peptide interactions with model Staphylococcus aureus membranes.

R. D. Harvey, T. T. Bui
School of Biomedical & Health Sciences, King´s College London, U.K.

Exposure to mildly acidic environments, such as those found on human skin and mucosal surfaces, increases the expression of the cationic phospholipid lysylphosphatidylglycerol (L-PG) in *Staphylococcus aureus*. Since L-PG has been shown to constitute up to 80% of the membrane phospholipids of *S. aureus*, it has been suggested that it plays a defensive role against the action of host cationic antimicrobial peptides (CAPs). In order to model the role of L-PG in defence against CAPs, vesicles composed of different mixtures of egg phosphatidylglycerol (EPG) and the monovalent cationic lipid dioleoyloxypropyl trimethylammonium chloride (DOTAP) have been used to examine the effect of changing surface charge upon peptide the membrane partitioning and folding.
Vesicles composed of various EPC:DOTAP mixtures were characterised by both size and zeta potential measurements. Using a lipid: peptide ratio of 100, the interaction between different CAPs and vesicle membranes were assessed by studying peptide binding and folding into ordered secondary structures; determined by measuring intrinsic tryptophan fluorescence and circular dichroism (CD), respectively. Vesicle membrane integrity was assessed by measuring the release of encapsulated carboxyfluorescein dye.
Vesicle zeta potentials were found to be significantly altered by only minor changes in lipid composition. For vesicles with neutral and negative zeta potentials, peptides were shown to readily bind and partition into the membrane, forming α–helices and causing significant membrane disruption. Vesicles with positive zeta potentials, however, showed a significant protective effect.

P-79

The action potential as a propagating density pulse and the role of anesthetics

T. Heimburg, A. D. Jackson
Niels Bohr Institute, University of Copenhagen, Denmark

The lipids of biological membranes display chain-melting transitions close to temperatures of physiological interest. During this transition the heat capacity, volume and area compressibilities all reach maxima. We show that this feature leads to the possibility of density pulse propagation in such membranes. In particular, if the membrane state is above the melting transition, the pulses will involve changes in lipid state. We discuss the propagating pulses in the context of several striking properties of nerve membranes under the influence of the action potential, including mechanical dislocations and temperature changes.
On the basis of the thermal properties of nerves we want to explain the influence of anesthetics on the threshold of nerve pulse excitation. We relate it to the famous but so far unexplained Meyer-Overton rule stating that the effectiveness of an anesthetic is closely linked to its membrane solubility. This approach is able to quantitatively explain the pressure reversal of anesthesia and the fact that inflamed tissue cannot be anesthetized.

P-80

Use of GBV-C/HGV synthetic peptides to select putative peptide inhibitors of the HIV fusion peptide

E. Herrera, M. Fernández, M. J. Gómara, I. Haro
Department of Peptide & Protein Chemistry, IIQAB-CSIC, Barcelona, Spain

Hepatitis G virus (HGV) and GB virus C (GBV-C) are two isolates of the same virus that belong to the Flaviviridae family. Recent studies have suggested that HGV/GBV-C infection in HIV-positive people is associated with slower progression of AIDS and prolonged survival (1-2). The mechanism by which the GBV-C/HGV virus has a "protective effect" in patients with HIV has still not been defined. Study of the interaction of the GBV-C/HGV and HIV viruses could lead to the development of new therapeutic agents for the treatment of AIDS.
The present work consists in studying the inhibitory capacity of the interaction and destabilisation process of membranes induced by the fusion peptide (FP) of the HIV glycoprotein, gp41, by different synthetic peptides corresponding to the envelope proteins of GBV-C/HGV using fluorimetric techniques. Peptides that inhibited the HIV-I FP in model membranes in an extent up to 50% have been subsequently characterized thermodinamically using ITC. Moreover, conformational analysis by CD and FTIR of peptide mixtures composed of E2 selected peptides and the FP gp41 have been carried out. Experiments to test the inhibition of cell-cell fusion promoted by gp41 expressed of cell surface are in progress. This work was supported by Project CTQ2006-15396-C02-01/BQU from the Ministerio de Ciencia y Tecnología (Spain).
1.Berzsenyi, M.D. et al., 2005, *J Clin.Virol.*, **33**, 257-266.
2.Kaiser, T. et al., 2005, *AIDS Rev.*, **7**, 3-12.

Abstracts

– Lipid biophysics –

P-81

ARF1-mediated actin polymerisation produces movement of artificial vesicles

J. Heuvingh, P. Chavrier, C. Sykes
Institut Curie, Paris, France

ADP-ribosylation factor (ARF 1) regulates vesicular trafficking and actin dynamics on Golgi membranes through the recruitment of various effectors including vesicular coats. ARF1-mediated actin assembly is supposedly based on a cascade that involves Cdc42 and its downstream effector N-WASP promoting Arp2/3 complex-dependent actin polymerisation. Proposed roles for the actin cytoskeleton on the Golgi complex include the control of Golgi organization and/or the regulation of vesicle formation and trafficking. Here we addressed the role of Golgi actin downstream of ARF1 using a biomimetic assay consisting of liposomes of defined lipid composition, carrying an activated form of ARF1 and incubated in cytosolic cell extracts. We observed actin polymerisation around the liposomes resulting in thick actin shells and actin "comet" tails. The assay was used to characterize the pathway, and confirmed a dependency on Cdc42 and its downstream effector N-WASP. In addition, the assay was inhibited by ARHGAP21, a GTPase Activating Protein (GAP) for Cdc42 interacting with and recruited to Golgi membranes by ARF1. This study therefore reveals that a biomimetic system can reproduce actin polymerisation driven by a complex multi-component signaling cascade. Moreover, we show that the liposomes move by forming actin comets that pushes them forward. Events of liposome separation were also observed and are based on the growth of actin filaments at the detaching liposome membrane. These findings suggest a role ARF1 mediated actin dynamics in detaching vesicles from the Golgi apparatus, and provides a model for the separation of vesicles out of their donor membrane.

P-82

Effects of plant sterols on structure and fluctuations of lipid membranes

A. Hodzic, M. Rappolt, H. Amenitsch, P. Laggner, G. Pabst
Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, Schmiedlstr. 6, A-8042 Graz, Austria

We have studied the concentration dependent influence of cholesterol, stigmasterol and sitosterol on the global structure and the bending fluctuations of fluid dimyristoyl phosphatidylcholine (DMPC) and palmitoyl oleoyl phosphatidylcholine (POPC) bilayers applying small-angle X-ray scattering, as well as dilatometry and velocimetry. Applying a previously developed global X-ray data analysis technique we find that the general effect of the plant sterols is different to that of cholesterol in terms of bilayer rigidification, lateral area per lipid, increase of the membrane thickness and decrease of water layer thickness. Additional ethyl groups and double bonds of stigma- and sitosterol cause less solubility and packing in the bilayer, which is evident for higher sterol concentrations and mono-saturated bilayer (POPC). We find for both lipids that cholesterol is most efficient in increasing bilayer thickness and decreasing the bilayer rigitification, followed by sitosterol and stigmasterol. Hence, it appears that some flexibility of the sterol hydrocarbon chain is needed in order to accommodate well within the lipid bilayer. In addition, we do not observe two populations of membranes within the nominal liquid-ordered/liquid-disordered phase coexistence regime of binary sterol/lipid mixtures. This lends further support to the idea of compositional fluctuations recently brought up by fluorescence microscopy experiments which contrasts the formation of stable domains within the miscibility gap.

This work was supported by the Austrian Science Fund (Grant No. P17112-B10).

P-83

Is there a special interaction between cholesterol and tryptophan?

A. Holt[1], A. Daily[3], R. W. H. M. Staffhorst[1], D. T. S. Rijkers[2], R. E. Koeppe[3], A. J. Killian[1]
[1]Chemical Biology, Utrecht University, Utrecht, NL, [2]Medicinal Chemistry, Utrecht University, Utrecht, NL, [3]Department of Chemistry & Biochemistry, University of Arkansas, Fayetteville, USA

Cholesterol can be found in almost all types of biological membranes which also contain a huge variety of proteins. Frequently these membrane proteins have Trp's located at the lipid/water interface. There are several indications for a special interaction between cholesterol and Trp. However, this has never been tested directly.
To investigate a possible preferential interaction between cholesterol and Trp we used a Trp-flanked alpha-helical transmembrane model peptide, called WALP23. This peptide was incorporated into phosphatidylcholine model membranes and the systems were investigated by solid-state ^{2}H-NMR and fluorescence spectroscopy. ^{2}H-NMR experiments using deuterated cholesterol incorporated into lipid bilayers, showed that the presence of WALP23 leads to a small but significant increase in quadrupolar splittings. However, control experiments with peptides of different composition showed that this could be ascribed to the membrane stretching effect of WALP23. In complementary experiments it was shown that addition of cholesterol had almost no effect on the quadrupolar splittings of a Trp-deuterated WALP23. Hence these experiments give no indication for preferential interactions between Trp and cholesterol. This was supported by fluorescence resonance energy transfer (FRET) experiments using a fluorescent cholesterol analogue, which showed that experimental FRET efficiencies match the simulated FRET efficiencies based on a random distribution of acceptors in the membrane.

P-84

The interaction study of phospholipase A2 with zwitterionic membranes.

W.-N. Huang, W.-G. Wu, Y.-H. Chen
Institute of Bioinformatics and Structural Biology, National TsingHua University, Hsinchu 30043, Taiwan

The binding orientations and penetration depths of PLA2s, however, had only been determined in highly negative charged membranes by using the method of infrared, electron paramagnetic resonance and fluorescence spectroscopy and proposed a tightly binding for simplifying orientation and penetration depth calculation. Nevertheless, it's well characterized that the cell membranes are abundance of zwitterionic lipids but only a few percentage of negative charged lipids. In this regard, the study is addressing the interaction of Taiwan cobra and bee venom PLA2 upon zwitterionic lipid membranes. To our surprises, PLA2s acted on 1,2-Di-O-tetradecyl-sn-glycero-3-phosphocholine (di-ether PC14), in the assay of monolayer binding experiments, would both decrease the amount of interfacial binding as well as the cross section area of enzyme (near zero) where the surface pressure of di-ether PC14 was reaching to a biomembrane equilibrant (around 30mN/m). This finding suggested that both PLA2s were sitting on the membrane surface instead of digging into the membrane. On the other hand, PLA2 will perturb the orientation of membrane fatty acid chain by the study of polarized-ATR-FTIR experiments and the carbon position dependent quenching of lysophosphatidylcholine membrane containing 10% phosphatidylcholines (Br2PC) brominated at the 6th to 12th position of the lipid acyl chains indicating that the membrane bound PLA2 was quenched maximally by 9, 10-Br2PC. Taking together, our results suggested that the PLA2 will bind to zwitterionic lipids peripherally instead of penetrate into the membrane and oblique the hydrophobic fatty acid chains of lipid.

Abstracts

– Lipid biophysics –

P-85

On the use of diffuse neutron reflectivity to probe domains in phospholipid layers

A. V. Hughes[1], P. Rees[2], M. R. Brown[2], S. J. Roser[3]

[1]ISIS, Rutherford Appleton Laboratory, Chilton, Didcot, UK, [2]School of Engineering, Swansea University, Swansea, UK, [3]Department of Chemistry, University of Bath, UK

Specular neutron reflectivity is widely used to probe the transverse structure of phospholipid layers. Potentially, off specular (or diffuse) scattering is a powerful technique which can yield information regarding the in-plane organisation of layers at interfaces, and so should allow the organisation of domains in mixed lipid systems to be investigated. However, the diffuse reflection of neutrons from phospholipid layers has not been reported previously. Here we report the first measurements of off-specular scattering from a phase separated lipid system.

Out test system is a mixed monolayer of deuterated DSPC and hydrogenated DMPC at the air-water interface. Partially deuterating the system provides a strong contrast between the two lipids, and diffuse scattering of reflected neutrons caused by the anisotropic phase separation is observed. The data is analysed using a statistical model in the Distorted Wave Born Approximation, and we are able to extract structural information about the layer, such as average domain sizes and separations. Future extensions of the work (including the potential benefits of the new instruments at ISIS /TS2 for studying domains) are discussed.

P-86

The study of dynamic and thermodynamic properties in bolaamphiphile membranes and their organisation

J. Jeftic, M. Berchel, L. Lemiègre, T. Benvegnu

ENSCR UMR CNRS 6226, Avenue du Général Leclerc, 35700 Rennes, France

We study the behaviour of bolaamphiphile molecules, a special class of lipids containing two polar heads connected by one or more hydrophobic segments. Our primary concern in this work is the organisation of these molecules. The bolaamphiphiles are present in the nature as the building units in membranes of archaebacteries. Since the archaebacteries are the organisms stable in extreme conditions, such as high salinity, elevated pressure, high or low temperature or strong acidity, we have undertaken an effort to synthesise their analogues (T. Benvegnu et al. *JACS*, **2004**, *126*, 10003.). The synthesis of bolaamphiphiles starting from biodegradable primary resources has been developed as well. Their organisation in aqueous solutions may be represented by mono- or multi- layered vesicles, lamellar phases or discs. This variety of structures is related to different thermodynamic and chemical parameters, such as temperature or concentration. The salt-influence has been observed as well, and we obtained the aggregates of 20 nm of diameter that align in structures resembling the pearls in a necklace in parallel, equidistant lines. We have studied the phase transitions of various bolaamphiphiles from stretched to fluid lamellar phase and from vesicles to discs. Our final aim is to use vesicles based on bolaamphiphiles in vectorisation for the drug delivery, so we are particularly interested in their thermodynamic stability, as well as in the membrane dynamics related to the efficiency of the vectors.

P-87

Domain coarsening and random domain patterns in ternary membranes

M. H. Jensen[1], E. J. Morris[2], A. C. Simonsen[3]

[1]Department of Physics, Boston University, Boston MA-02215, USA, [2]SEAS, Harvard University, Cambridge, MA-02138, USA, [3]MEMPHYS, University of Southern Denmark, Odense, Denmark

A number of morphological and statistical aspects of domain formation in a single and double supported ternary membrane have been investigated. Ternary membranes composed of an unsaturated and a staurated phospholipid and cholesterol display macroscopic phase separation in two fluid phases. They are widely used as raft models. We find that membrane interactions with the support surface can have a critical influence on the domain shapes. Combined AFM and fluorescence microscopy demonstrates small (500 nm) irregular domains and incomplete formation of much larger round domains. The interaction-artefacts can be effectively removed by employing double supported membranes under physiological salt concentrations. These membranes are prepared by a recently developed method involving hydration of spincoated lipid films[1]. The planar membrane geometry allows a quantitative characterization of domain coarsening upon rapid cooling into the coexistence region. We determine a domain growth exponent α=0.31 in close agrement with the theoretical value of 1/3. Analysis of the spatial domain pattern in terms of Voronoi polygons demonstrates a close similarity to equilibrated cellular structures with a maximized configurational entropy.

[1] A. C. Simonsen and L. A. Bagatolli. Langmuir, 20:9720-9728 (2004)

[2] Mikkel H. Jensen, Eliza J. Morris, A. C. Simonsen, Langmuir (submitted, 2007)

P-88

Inhibition of the inverse ribbon phase by sugars in dry DOPC/DOPE mixtures: a synchrotron SAXS study

B. Kent[1], G. Bryant[1], C. J. Garvey[2], D. Cookson[3]

[1]Applied Physics, School of Applied Sciences, RMIT University, Melbourne, Australia, [2]Australian Nuclear Science and Technology Organisation, Menai, Australia, [3]Australian Synchrotron Research Program, Argonne, USA

Severe dehydration causes irreparable changes to biological membranes, such as the transition from stable bilayer phases to lethal non-bilayer phases. However, there are a number of organisms which have evolved mechanisms to avoid damage during dehydration. One of these mechanisms is the accumulation of small solutes, which have been shown to preserve membranes by inhibiting deleterious phase changes at low hydration. The effect of sugars on both the phase behaviour and transition kinetics of membranes is therefore of primary importance in understanding freezing and dehydration damage.

We report on an investigation of the effects of sucrose on the phase behaviour of dehydrated DOPC:DOPE mixtures using small angle synchrotron X-ray scattering and DSC. As the temperature is increased from -10°C to 80°C the pure lipid system undergoes a transition from a bilayer fluid lamellar phase (L_α) to an inverse hexagonal phase (H_{II}) via an intermediate orthogonal inverse fluid ribbon phase (P_α). Adding sucrose to the system is observed to prevent the formation of the P_α phase - instead the samples go directly from L_α to H_{II}. Increasing the sucrose ratio was found to increase the stability of the bilayer L_α phase. In this paper we discuss the effects of sugars on both the structure of each phase, and on the kinetics of the phase transitions. We discuss the importance of these results to our understanding of membrane damage during freezing and dehydration.

Abstracts

– Lipid biophysics –

O-89

The dynamics of mitochondrial cristae – a study involving GUVs and local pH gradient modulation

N. Khalifat[1], N. Puff[1], S. Bonneau[2], M. I. Angelova[1]

[1]UPMC, INSERM UMR 538, 75012 Paris, France, [2]UPMC, CNRS UMR 7033, 91030 Evry, France

The mitochondrial inner membrane presents membrane folds termed cristae. The electron transport chain pumps protons from the matrix, establishing a proton-motrice force across inner membrane. Protons are driven back into the matrix through the F_0F_1-ATPase allowing the conversion of ADP to ATP. Mitochondria inner membrane has similar composition and function in eukaryote cells and presents important polymorphism. In certain cases the morphology looks like extreme states of fusion or fission of inner membrane. The dynamics of the inner membrane topology might involve regulation mechanisms as far as membrane functions might be affected by changes in membrane shape, and as the electron chain proteins and F_0F_1-ATPase are localised in the cristae. On the other hand, localized proton flow could modify local physical properties of the lipid membrane and therefore influence membrane mechanics and shape. It became clear that the cristae are not random folds but real internal compartments originating at cristae junctions. Is the dynamics of inner membrane morphology ruled only by specific mitochondrial proteins, or, lipid mediated processes may take place as well? In this work we use giant unilamellar vesicles (GUVs) made of cardiolipin and phosphatidylcholine, optical microscopy and micropipette technique for modelling cristae dynamics. We show that: (i) creation of local pH gradient induces cristae–like membrane invaginations, and (ii) the morphology of these invaginations is dynamic. Depending on the local pH gradient, the induced invaginations can progress to fission, or, go rearwards and disappear.

P-90

Polyphenol compounds as effective antioxidants

H. Kleszczyńska[1], D. Bonarska-Kujawa[1], A. Włoch[1], B. Tomicki[1], J. Oszmiański[2]

[1]Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences Norwida 25, 50-375 Wrocław, Poland, [2]Department of Fruits and Vegetable Technology, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland

It was studied the effect of polyphenols extracted from the 'Idared' apple, skullcap (*Scutellaria baikalensis*) and strawberry on the degree of lipid oxidation in the erythrocyte membrane. The aim of the studies was to determine the antioxidative activity of the mentioned extracts with respect to membrane lipids, having in view their practical application for protecting biological membranes against oxidation. The studies were carried out on erythrocyte ghosts, oxidized by UV-B radiation. The substances studied were obtained from the Department of Fruit and Vegetable Technology. The studies showed a very high antioxidative activity of the substances mentioned, their amounts in erythrocytes suspension varied, being in the range 0.005 mg/ml to 0.1 mg/ml. The substances studied at highest concentration (0.1 mg/ml) inhibited practically in full the oxidation of membranes irradiated with a UV lamp for 60 minutes, though the kinetics of the protective action varied. The present studies may bring about a wider range of the extracts application, especially with respect to biological systems that can thus be protected against oxidation stress.

The work was sponsored by the Polish State Committee of Scientific Research, grant no. 2 P04G 088 27.

P-91

Probing lipid rafts in model and cellular membranes with hydration sensitive dyes

A. S. Klymchenko, G. M´Baye, V. V. Shynkar, D. A. Yushchenko, S. Oncul, G. Duportail, Y. Mély

Photophys. Interactions Biomoléculaires, UMR 7175 du CNRS, Fac. Pharmacie, Université Louis Pasteur, 67401, Illkirch, France

Lipid domains (rafts) are believed to be involved in a variety of biomembrane functions. Herein, we studied raft forming systems using our recently developed 3-hydroxyflavone fluorescent probe (F2N8), which exhibits high sensitivity to hydration of lipid bilayers (Klymchenko et al, BBA 2004, 1665, 6). The results obtained in vesicles composed of sphingomyelin or DPPC with varying cholesterol concentration and temperature suggest the strong dehydration of the raft phase compared to gel and fluid phases. Surprisingly, the fluidity monitored by TMA-DPH does not correlate with the changes in the hydration, especially when raft and gel phases are compared. Thus, gel and raft phases showing similar fluidity differ significantly by the hydration level. The low hydration of the raft phase is probably due to high density packing of the lipids with cholesterol, which decreases the void space for water molecules. These observations shed light on our recent studies of apoptotic transformation of the cell plasma membrane using an analog of F2N8 (F2N12S), which selectively stains its outer leaflet (Shynkar et al, J. Am. Chem. Soc. 2007, 129, 2187). We observe that the outer membrane leaflet of normal cells exhibits remarkably low hydration, similar to sphingomyelin-cholesterol mixtures. In apoptotic cells the hydration appears significantly larger, suggesting that the apoptosis induced loss of the transmembrane lipid asymmetry is accompanied by the loss of the raft organization at the plasma membrane.

P-92

Molecular dynamics simulations of lipid vesicle fusion in atomic detail

V. Knecht[1], S.-J. Marrink[2]

[1]Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany, [2]Department of Biophysical Chemistry, University of Groningen Nijenborgh 4, 9747 AG Groningen, The Netherlands

The fusion of a membrane-bounded vesicle with a target membrane is a key step in intracellular trafficking, exocytosis, and drug delivery. Molecular dynamics simulations have been used to study lipid vesicle fusion in atomic detail. Starting from a preformed stalk between the outer leaflets of a vesicle and its periodic image, a hemifused state formed within 2 ns and remained stable on a timescale of up to 11 ns in six independent simulations. Forcing a single lipid into the interior of the hemifusion diaphragm induced the formation and expansion of a fusion pore on a nanosecond timescale. This work opens the possibility to investigate in detail how vesicle fusion is influenced by lipid composition and (fusion) peptides.

Abstracts
– Lipid biophysics –

P-93

The effects of membrane stored curvature elastic stress and charge upon CCT

J. M. Knott, O. Ces, R. H. Templer, K. H. Parker, R. Woscholski
Imperial College London, Exhibition Road, South Kensington Campus, SW7 2AZ, London, U.K.

CCT (CCT:CTP phosphocholine cytidyltransferase) is a rate limiting enzyme that catalyzes the biosynthesis of one of the most abundant membrane lipids, phosphocholine (PC). CCT contains four discrete domains with domain M exhibiting an α-helical conformation when it is bound to the membrane[1]. Previous evidence has suggested that the stored curvature elastic stress in the membrane modulates the activity of the enzyme, in a feedback mechanism[2,3]. When levels of stored of stored curvature elastic stress are high, the α-helix inserts its hydrophobic portion into the bilayer. This causes the nearby lipids to splay and relaxes the stress. Furthermore, because CCT catalyzes the production of the Type II lipid PC, this will also aid the relaxation of the bilayer stress and the membrane can return to its original state. In this work, the partition coefficients of a 33 residue portion of domain M to vesicles with varying levels of SCES and charge were determined through an o-phtaldialdehyde assay[4]. We confirm that the insertion of domain M is controlled by stored curvature elastic stress and show that the effects of stress and charge upon binding are surprisingly comparable.

1. Johnson, J.E., Cornell R.B, *Biochemistry*, 1994, **33**, 4327
2. Attard, G.S., Templer, R.H., Smith, W.S.,et al, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 9032
3. Ces, O., Mulet, M. *Signal Transduction*, 2006, **6**(2), 112
4. Johnson, J.E., Rao, N.M., Hui, S.W, Biochemistry, 1998, **37**, 9509

P-94

Molecular dynamics study of a lipid particle: clues to the atomic scale structure of lipoproteins

A. Koivuniemi[1], M. Heikelä[2], I. Vattulainen[3], P. T. Kovanen[1], M. T. Hyvönen[1]
[1]Wihuri Research Institute, Helsinki, Finland, [2]Laboratory of Physics and Helsinki Institute of Physics, Helsinki University of Technology, Finland, [3]Institute of Physics, Tampere University of technology, Finland

Lipoproteins are water-soluble globular particles, which transport water-insoluble lipids in the circulating blood. The particles are composed of a surface containing phospholipids and proteins, and of a hydrophobic core consisting of cholesteryl esters and triglycerides [1]. Low density lipoproteins (LDLs) and high density lipoproteins (HDLs) play major roles in the development of atherosclerosis, the former ones promoting and the latter ones preventing its development. We have previously constructed a model for the core of lipoprotein particles containing cholesteryl oleates (COs) [2]. The model studied through atom-scale molecular dynamics simulations clarified the structural and dynamical properties of CO molecules in the lipoproteins [2]. In this study, 35 CO molecules combined with 180 palmitoyloleoylphosphatidylcholine (POPC) molecules were used as a model to study the organization of lipids in lipoproteins. Results indicate that, depending on the solvation degree of COs into the POPC membrane, complexes of CO and POPC molecules form either discoidal or spheroidal lipid particles. In both cases, the structural and dynamical properties of the CO and POPC molecules were compared with those of pure CO and pure POPC systems.

[1] Hevonoja et al. Biochim. Biophys. Acta 1488 (2000) 189
[2] Heikelä et al. Biophys. J. 90 (2006) 2247

P-95

Band-3 clustering agents induce phosphatidylserine externalization on erythrocyte surface.

A. Koshkariev, S. Yedgar, G. Barshtein
Department of Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

It is known that the phagocytic removal of senescent or damaged red blood cells (RBC) and their adherence to endothelial cells (EC), are induced by band-3 clustering or phosphatidylserine (PS) translocation at the outer leaflet of RBC membrane. Both band-3 clustering and PS exposure are known to increase with RBC aging, and in the various pathologies, such as sickle cell anemia, malaria, and thalassaemia. A widely used procedure in the study of band-3 clustering has been the treatment of RBC with acridine orange (AO) and $ZnCl_2$, as it was assumed to be a specific inducer of band-3 clustering. The present study was undertaken to examine the influence of band-3 clustering agents on PS level at the RBC surface.

PS externalization was determined by binding of fluorescently-labeled annexinV to RBC surface.

We show that band-3 clustering agents strongly induce translocation of PS to RBC surface. The mechanism of AO-induced PS externalization is probably different from calcium-dependent scrambling.

According to our data, treatment of RBC with band-3 aggregating factors very effectively induces translocation of PS to the cell surface. It is possible that band-3 clustering and PS translocation are interdependent, but this interrelation has yet to be explored. On these grounds we may assume that membrane proteins aggregation (especially band-3) plays one of the central roles in the control of membrane phospholipid distribution.

P-96

Binding of β2-glycoprotein I to phospholipid vesicles and its effect on their shape

J. Kovačič[1], G. Anderluh[2], B. Božič[1], S. Čučnik[3], J. Derganc[1], J. Majhenc[1], B. Rozman[3], S. Svetina[1]
[1]Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia, [3]Department of Rheumatology, University Medical Centre, Ljubljana, Slovenia

β2-glycoprotein I (β2GPI, also known as apolipoprotein H) is a 50 kDa plasma glycoprotein which binds to negatively charged phospholipids. A patch of positively charged amino acid residues contacts anionic phospholipids via electrostatic interactions, and a short amino acid loop of the protein is inserted into the outer phospholipid layer via hydrophobic interactions. β2GPI has different biologically relevant roles amongst which is its involvement in autoimmune disease antiphospholipid syndrome (APS). It mediates the binding of antiphospholipid antibodies to the membrane, therefore its binding specificities are important for the pathogenesis of APS. We studied the binding kinetics of β2GPI of different concentrations when introduced to large phospholipid vesicles of different sizes. Vesicle membranes were composed of 20 % POPS and 80 % POPC. Binding curves (sensorgrams) obtained by surface plasmon resonance (SPR) were analyzed by curve-fitting with single exponential function. Association and dissociation phases were fitted separately. The obtained data support the interpretation of the β2GPI induced budding of giant phospholipids vesicles, observed by an optical microscope, on the basis of the bilayer-couple mechanism.

Abstracts

– Lipid biophysics –

P-97

Changes in membrane order parameters of cultured cells by exposure to 2.45 GHz microwaves

E. Kovacs[1], T. Savopol[1], D. Martin[2], N. Iacob[2], S. Miclaus[3], L. Bajenaru[1]

[1]Biophysics and Cell Biotech. Dept., Carol Davila Medical University, POBox 35-43, Bucharest, Romania, [2]National Institute for Lasers, Plasma and Radiation Physics, Bucharest, [3]Land Forces Academy, Sibiu, Romania

Cellular effects of microwave irradiation were reported by many laboratories but the detailed biophysical mechanisms for these effects are not currently available and experiments on in vitro models are expected to offer the keys to understanding the nature of the so called non-thermal effects.
In this work we study changes in two membrane biophysical parameters of cells exposed to microwaves in order to analyse the specific interaction mechanisms.
1/ changes of *lipid packing* in *membrane bilayer* formally reflected in a fluorescent marker mobility. These changes would shift the temperature dependence curves and phase transition temperature in irradiated versus control samples as shown by fluorescence depolarization recordings.
2/ change in *generalized membrane polarization* which is reflected in the fluorescent emission of Laurdan – a fluorescent dye, sensitive to polarization of hydrophilic environment within the lipid bilayer.
Our experiments provide the evidence that in a living cell membrane as well as in a simple bilayer systems (liposomes) microwave irradiation induces a change in local order of lipid packing (membrane fluidity) and in orientation of polar molecules. These changes are consistent with many reported cellular effects of 2.45 GHz.

O-98

Point-like protrusion as a pre-stalk intermediate in membrane fusion pathway

M. Kozlov
Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Israel

The widely accepted pathway of membrane fusion begins with the fusion stalk representing the initial intermediate of hemifusion. The lipid structures preceding hemifusion and their possible influence on fusion kinetics were not addressed. Here, we suggest the point-like protrusion as a pre-stalk fusion intermediate, which has energy lower than that of stalk and, therefore, does not limit the fusion rate. We demonstrate that by calculating the energy of the point-like protrusion, which depends on the lipid monolayer elastic parameters and the strength of the inter-membrane hydration repulsion. The point-like protrusion completes the fusion-through-hemifusion model of membrane merger.

P-99

From playing blocks to membrane pore modeling

K. Kubica, M. Kotulska
Departament of Biomedical Engineering and Instrumentation, University of Technology, Wroclaw, Poland

The basis of Monte Carlo simulation technique is defining energy of an examined system. To do this, the set of possible configurations has to be precisely described. Our Monte Carlo studies on lipid membrane properties, based on modified Pink's model, permitted to examine the mechanism of induction of the electric field-driven defects, which we called the pre-pore excitation. We observed that the electric field above some critical value causes slight changes in lipid chain conformations, increasing the packing density of one lipid layer and decreasing it in the other layer. If our next aim is examination of the pore appearance, its enlargement and stabilization under some conditions, we should define the pore structure. Our model is based on a triangular lattice where one lipid molecule is assigned to two lattice nodes. To study the influence of environmental factors on the pore appearance, we should define how to construct a pore connecting two parallel triangular nets, keeping the basic net element, i.e. the equilateral triangle. Next, we have to show that we are able to enlarge the pore size without changing the number of lipids that constitutes the examined system. Using children blocks we want to show the transformation leading to the pore formation between two parallel networks of triangular symmetry and the possibility to its enlargement. By MC technique we will estimate the stabilization energy of such a pore.

P-100

Monoelaidin-water phase behaviour with temperature.

C. V. Kulkarni, O. Ces, R. H. Templer
Membrane Biophysics Group, Dept. of Chemistry, Imperial College, SW7 2AZ, London, United Kingdom.

Monoacylglycerols are known to form a variety of mesophases when mixed with a range of water compositions. These include the lamellar, hexagonal and cubic phases among the others. A special class, known as bicontinuous cubic phases are based on the mathematical surfaces called IPMS or TPMS (Infinitesimally or triply periodic minimal surfaces). Commonly observed bicontinuous cubic phases are P (Primitive), D (Diamond) and G (Gyroid) types, which have the corresponding space groups Im3m, Pn3m and Ia3d represented respectively *as* $Q^{II}{}_{229}$, $Q^{II}{}_{224}$ and $Q^{II}{}_{230}$.
Depending on the amphiphilicity and shape of the molecule of that lipid, these mesophases are observed to be stable individually or in the coexistence, at different temperatures and hydrations. Monoolein (*cis* double bond) with 18-carbons shows two of the above cubic phases, namely Ia3d and Pn3m. We have found that, another conformer of monoolein i.e. monoelaidin (*trans* double bond) shows each of the three commonly observed cubic bicontinuous phases with water.
In this work we present the various mesophases along with the three bicontinuous cubics, observed with the lipid, monoelaidin, by varying the water and temperature. We have characterized them by X-ray diffraction.

Abstracts

– Lipid biophysics –

P-101

Antimicrobial peptide interactions with model lipid membranes

M. D. Lad, R. J. Green
Reading School of Pharmacy, University of Reading, U.K.

Antimicrobial peptides are small, predominantly cationic peptides that selectively interact with biological cell membranes leading to cell death. This peptide-lipid interaction is of biological and pharmaceutical importance, as these peptides possess the ability to be potent antibiotics. However, the selectivity of various peptides towards different cell membrane types varies widely and is not clearly understood. The interaction of antimicrobial peptides of varying lipid selectivity (melittin (MLT), magainin 2 (MGN) and cecropin P1 (CECP1)) with mixed lipid layers of phospholipids, sphingolipids and cholesterol were studied in order to investigate lipid binding selectivity and modes of interaction. Through the combined use of three complementary methods, surface pressure measurements, external reflectance Fourier transform infrared (ER-FTIR) spectroscopy and neutron reflectivity, peptide binding to lipid monolayers was defined in terms of adsorbed layer structure and peptide conformation. Differences in the mode of binding of the three peptides was linked to the charge distribution throughout their primary sequence and shown to relate to their different behaviours in vivo. By varying the lipid layer composition, different binding behaviour was observed that may link to the cell selectivity of these peptides and further reveals the important role of lipid structure and composition in the biological membrane structure.

O-102

Cholesterol sulphate affects the mixing properties of the main lipid components of stratum corneum

M. Lafleur, M. Arseneault, G. Bastiat
Dept. of chemistry, Université de Montréal, Canada

Cholesterol sulphate (Schol) is an essential component of the lipid matrix of stratum corneum (SC), the top layer of the epidermis. Moreover a change in its proportion can lead to severe skin pathologies. Using vibrational and ^{2}H-NMR spectroscopy, we show that this anionic sterol has a profound influence on the mixing properties of the main lipid components of SC. First the presence of Schol modifies the binding of Ca^{2+} to the lipid matrix. In the absence of Schol, a complexation involving Ca^{2+} and free fatty acids is observed and these complexes are thermostable, leading to an extensive phase separation over a wide temperature range. Schol changes the nature of the Ca^{2+}-binding site, inhibits the complexation with fatty acids, and promotes lipid mixing. We also show that it is possible to form, with palmitic acid - sterol mixtures, fluid lamellar phases where the sterol content is very high. The stability of these self-assembled bilayers is found to be pH dependent and this property is controlled by the cholesterol/Schol molar ratio. This phase behavior is understood in terms of the balance between the intermolecular interactions between the constituting species. The large unilamellar vesicles prepared from these mixtures can constitute versatile vectors for pH-triggered release. The balance in the equilibria involving Ca^{2+}, cholesterol, cholesterol sulphate, and fatty acids is proposed to have an impact on the organization and the function of the epidermis.

P-103

Phase State depending Current Fluctuation in Lipid Membranes

C. T. Leirer[1], A.-L. Idzko[2], T. Heimburg[1], A. Wixforth[1], M. F. Schneider[1]
[1]Institut für Physik, Universität Augsburg, D-86159 Augsburg, Germany, [2]Niels Bohr Institut, University of Copenhagen, DK-2100 Copenhagen, Denmark

The permeability of lipid membranes has often been discussed as a potential control mechanism for drug delivery systems. However, until now the mechanism of ion or drug transport through the membrane has not been resolved.
Here, we present the impact of the lipid phase state on the permeability of mixed phospholipid membranes. In addition to an overall increase in membrane permeability, we found channel like current fluctuations in the phase transition region. Above and below the phase transition region, in the fluid as well as in the gel state, the current fluctuations exhibit no distinct on-off–like pattern but revealed individual time scales in the millisecond range. This behaviour clearly changes when entering the phase transition regime where channel-like on-off behaviour with increased time scales ($\sim$100ms) has been observed. The dependence of these time scales on the heat capacity of the system is discussed in terms of their relation to the relaxation times in the membrane. Furthermore, the onset (voltage threshold) of current fluctuations and its relation to the initial membrane resistance and phase state is resolved.

P-104

A Novel Iron Chelator with Anti-microbial Activity: Insights on its Interaction with Membrane Models

A. Leite[1], A. Nunes[1], P. Gameiro[1], B. Castro[1], M. Rangel[2]
[1]REQUIMTE, Departamento de Química, Faculdade de Ciências, Universidade do Porto, 4069-007 Porto, Portugal, [2]REQUIMTE, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4099-003 Porto, Portugal

A novel iron(III) chelator synthesized in our laboratory has proved to be effective in inhibiting the growth of *Mycobacterium avium* inside mouse bone marrow derived macrophages. Apart from high affinity towards iron, the compound seems to possess a key molecular structure that allows its permeation through biological membranes. With the purpose of establishing the ideal molecular structure for targeting infection, we are presently exploring its physicochemical properties and its partition on models of biological membranes. To get insight on the partition and location of this new compound fluorescence spectroscopic studies are being performed in LUV's with different hydro/lipophilic characteristics. In this work we report the determination of partition coefficients of this compound and of Rhodamine in DMPC, and DMPG liposomes. The results show that the new compound strongly interacts with the lipid phase and comparing the obtained values of the partition constants of both compounds it seems that this new molecular structure promotes a better partition into the lipid phase. These results also suggest that further studies with different types of lipids are necessary and will help to separate surface effects and lipophilicity effects.
Acknowledgements Partial financial support was provided by "Fundação para a Ciência e Tecnologia" through Project (POCI/QUI/56214/2004); AN and AL thanks FCT for PhD grants (SFRH/BD/9331/2002), (SFRH/BD/30083/2006).

Abstracts

– Lipid biophysics –

P-105

Can computational modelling help to improve the properties of antimicrobial peptides?

S. Linser[1], S. S. Funari[2], D. Bechor[3], N. Ben-Tal[3], R. Willumeit[1]
[1]GKSS Research Center, Max-Planck-Str. 1, Geesthacht, Germany, [2]MPI-KGF c/o Hasylab@DESY, Notkestr. 85, Hamburg, Germany, [3]Tel Aviv University, Department of Biochemistry, Tel Aviv, Isreal

The uprising resistance of pathogenic bacteria against treatments with conventional antibiotics is a world wide problem and emerged an acute search for alternatives. One class of promising alternatives are naturally occurring antimicrobial peptides, which belong to the innate immune system of many different species. These peptides act by direct interaction with the membranes of their target cells and can thereby be distinguished from antibiotics. Another important feature of antimicrobial peptides is their high affinity for bacteria and fungi in contrast to mammalian cells. In our work we tested the activity of the small antimicrobial peptide NK-CS (27 amino acids), and several modifications thereof as well as a peptide developed by computational modelling. All peptides are active against *Escherichia coli* (Gram negative) and *Staphylococcus carnosus* (Gram positive) bacterial cultures, but the haemolytic properties against human red blood cells were found to be absolutely poor and indicated the peptides' selectivity. Subsequently we investigated the interaction of the peptides with different model membranes by small angle X-ray scattering. We observed an influence of all tested peptides on the inverse hexagonal phase transition of phosphatidylethanolamine lipids. The inverse hexagonal phase transition temperature was increased by the peptides and this promotes a positive curvature of the membranes. We assume that this curvature finally leads to the disruption of the model membranes.

P-106

Lipid bilayer permeation studies using Tb(III) complexes as a probe: thermodynamics and kinetics

D. Lombardi, H. Wunderli-Allenspach, S. D. Kraemer
ETH Zürich, Switzerland

A liposomal assay for the direct measurement of the kinetics of lipid bilayer permeation of aromatic carboxylic acids (ACAs) was introduced in a recent study [1]. The permeation rate constants are determined from the increase in luminescence of intraliposomal Tb(III) which gets ligated by permeating ACAs.
In this work equilibria and kinetics of the complexation of Tb(III) by 2-hydroxynicotinic acid (2-OH-NA) are performed in two different buffer systems at pH 6.5 by means of a luminescence spectrofluorimeter equipped with a stopped flow device. The 1:1 complex between Tb(III) and 2-OH-NA has a K_D value around 9.1×10^{-7} M in MOPS and around 2.1×10^{-5} M in TRIS.
The van't Hoff analysis reveals that the buffer affects the free energy variation (ΔG) of the reaction between 2-OH-NA and Tb(III) mainly influencing the enthalpic term (ΔH). The presence of liposomes decreased the apparent complex affinity.
The permeation kinetics of 2-OH-NA were fitted with a bi-exponential function suggesting a two phase reaction. The analysis of the kinetic experiments for the complexation of intraliposomal Tb(III) suggested that the buffer has no influence on the fast phase of permeation kinetics but it has an influence on the slower phase.
The temperature dependence of the permeation kinetics followed Eyring's equation. Fitting the data with Eyring's equation the variation of the enthalpy of activation (ΔH*), the variation of entropy of activation (ΔS*), the free energy of activation change (ΔG*) and the activation energy (E_a) were obtained.
[1] Thomae AV, Wunderli-Allenspach H, Krämer SD. *Biophys. J.* 2005; 89:1802-1811.

P-107

Effect of lipid membrane modulation on integral membrane protein activity

M. Lorch, R. Batchelor
University of Hull, Hull, HU6 7RX, U. K.

The activity of integral membrane proteins is known to be affected by the behaviour of the surrounding lipid bilayer. In order to observe the effect of the bilayer on embedded proteins, photoisomerisable azobenzene molecules were synthesised as lipid analogues for incorporation into lipid vesicles. Two synthetic pathways were followed. The effect of photoswitching between the azobenzene cis and trans states on the lipid bilayer was investigated using magic angle spinning NMR spectroscopy to show order parameters and location probabilities of the azobenzene group. E.coli diacylglycerol kinase was expressed and incorporated into the azobenzene:lipid vesicles. The activity of DGK on photoswitching between azobenzene cis and trans states was assessed using enzyme activity assays.

P-108

A single vesicle study of the interactions of melittin with phospholipid membranes

M. Mally, J. Majhenc, S. Svetina, B. Žekš
Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

In order to study the interaction of antimicrobial peptide melittin (MLT) with lipid membranes we employ direct optical observation of single giant (cell-size) phospholipid vesicles with a phase-contrast microscope. On the basis of the observed changes of the vesicle we established that there are two mechanisms of transport of the solvent molecules: in addition to the transport of molecules through the MLT-induced pores there is also a transport due to osmotic pressure induced periodical bursts of the vesicle membrane. The time intervals between the bursts are decreasing with time, yielding that the MLT induced membrane permeability for solvent molecules increases with time. While studying the effects of MLT at different concentrations the methodology allows us to observe different phenomena from slight perturbation of the membrane at low concentrations to disintegration of the vesicle at high concentrations of MLT. In a proposed theoretical model the observed vesicle responses are explained for the entire span of MLT concentrations. The underlying mechanisms of MLT action are deduced: transmembrane positioning and pairing of MLT, the lipid flow from the outer to the inner membrane leaflet induced by MLT translocation, formation of pores and the consequent transport of small molecules through the membrane. On the basis of the theoretical analysis the role of MLT pairs is stressed and it is suggested that MLT pairs translocate the membrane already at low MLT concentration where pores are not yet formed. By using fluorescence microscopy it is attempted also to confirm this hypothesis experimentally.

Abstracts

– Lipid biophysics –

O-109

Comparisons of the phase properties of dispersions of cholesterol- and epicholesterol/DPPC mixtures

D. A. Mannock, M. Lee, R. N. McElhaney
Department of Biochemistry, University of Alberta, Edmonton, AB, T6G 2H7, Canada

We have studied the thermotropic phase behaviour of dipalmitoylphosphatidylcholine (DPPC) dispersions containing different concentrations of cholesterol (*chol*, 3β-OH) or epicholesterol (*epichol*, 3α-OH), using high-sensitivity differential scanning calorimetry. On heating, samples containing either sterol, the pretransition centered at ∼35°C is gradually broadened and shifted to lower temperature, disappearing at 10 (*chol*) or 7 (*epichol*) mol%. The chain-melting phase transition (MT) initially seen at 41.9°C also increases in width and can be deconvolved into a sharp and a broad component in both sterol/phospholipid systems. In *chol*/DPPC mixtures, the sharp component is visible over the concentration range from 3-20 mol%, whereas in *epichol*/DPPC mixtures the sharp component persists up to 30 mol%, suggesting that *epichol* is less soluble in the DPPC bilayer than is *chol*. Plots of the MT phase transition temperature (T_m), enthalpy (ΔH) and peak half width ($\Delta T_{1/2}$) for both deconvolved components for each sterol/phospholipid system show several similarities, but for the sharp component in the *epichol* system, the T_m and ΔH are lower and the $\Delta T_{1/2}$ higher above a concentration of 7 mol% sterol, whereas the corresponding parameters for the broad component are all lower than those seen in the *chol*/DPPC mixtures up to a concentration of 40 mol%. Above this concentration, the ΔH of the *epichol* system exceeds that of the *chol*/DPPC mixtures which show no MT endotherm at concentrations $\geq$ 50 mol%. This observation also supports the idea that *epichol* is less soluble in DPPC than is *chol*.

P-110

Building a Protocell

S. S. Mansy, J. W. Szostak
Massachusetts General Hospital & Harvard Medical School, Boston, Massachusetts, U.S.A.

Simple, single-chain, prebiotic-like molecules readily assemble into vesicle structures that display a variety of characteristics necessary to sustain biological reactions. For example, these vesicles are impermeable to large molecules, such as strands of DNA, permeable to small molecules, such as nucleotides and sugars, and are heat stable. While the vesicles do not rupture at high temperatures, they become progressively more permeable as the temperature is raised. Therefore, selective nutrient exchange can occur by temperature fluctuations. The permeability properties of the vesicle system can be further tuned by modulating acyl chain fluidity and amphiphile head group size. All of these characteristics are the result of purely physical forces arising from the amphiphiles themselves and do not depend on membrane protein components. The prebiotic plausibility of the system was tested by reconstituting non-enzymatic nucleic acid polymerization reactions within the vesicles.

P-111

Membrane fusion induced by E1(70-87) peptide from E1 structural protein of Hepatitis G virus

M. Massanés[1], M. Muñoz[1], M. A. Busquets[1], M. A. Alsina[1], I. Haro[2], M. Pujol[1]

[1]Department of Physical Chemistry. IN2UB. Barcelona University. Spain., [2]Department of Peptide and Protein Chemistry. IIQAB-CSIC. Barcelona. Spain

Hepatitis G virus (GBV-C/HGV) is frequently associated with hepatitis C virus (HCV) infection. People co-infected with GBV-C/HGV and human immunodeficiency virus (HIV) has delayed progression of the HIV disease. The mechanism how GBV-C/HGV could inhibit the progression of AIDS needs to be defined. At this work, we report the effect of E1(70-87) synthetic peptide derivative of a continuous epitope from the E1 capsid protein of GBV-C/HGV on lipid bilayers by fluorescence techniques: resonance energy transfer (RET) and ANTS/DPX fusion assay to know the possible fusion properties of it. The complete peptide sequence is ^{69}LGSLYGPLSVSAYVAGIL^{88}. Small (SUVs) and large (LUVs) unilamellar vesicles prepared by sonication and extrusion respectively were used [(POPC-SM-DPPE-PS) (40:33:12:15)]. The vesicle mixture used for RET contained 0,6% of NBD-PE or Rh-PE co-dispersed with the unlabelled lipids to obtain the desired lipid composition. Excitation and emission fluorescence spectra from NBD were registered. The results show that the sequence is able to cause destabilization of lipid membrane at the pH range from 7,4 to 4,0. This effect is greater as the more acidic is the pH medium. This conclusion is supported by the insertion of E1(70-87) into lipid monolayers of the same composition spread at the air/water interface.

P-112

Molecular modeling of syringomycin-E – membrane interactions

E. Mátyus[1], D. P. Tieleman[2]

[1]Institute of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, [2]Departement of Biological Sciences, University of Calgary, Calgary, Canada

Syringomycin-E (SR-E) is a cyclic lipodepsipeptides produced by certain strains of the bacterium *Pseudomonas syringae pv. syringae*. It shows inhibitory effects against many fungal species, including human pathogens. Its primary biological target is the plasma membrane, where it forms channels comprised of at least six SR-E and 40 lipid molecules. The high-resolution solvent structure of SR-E and the structure of the channels are currently not known. The studies of SR-E channel structure may serve better understanding its biological activity. In our work we investigated in atomic detail the molecular features of SR-E in hydrophilic, hydrophobic and lipid bilayer environment by molecular dynamics simulation (MD). As a first step we built a model of peptide and examined its structure in water and octane in 200 ns MD simulations including experimental NMR NOE data. We determined structural preferences and conformational flexibility of SR-E in both solvent in particular, the importance of side-chain interactions in determining peptide stability. The obtained three-dimensional structures are used as a basis to investigate the interactions of SR-E with lipid membranes, and the structure of channels formed by SR-E. To study the influence of the lipid charge and shape on peptide-lipid interactions and on pore formation we built DOPE, DOPS and mixed DOPE/DOPS bilayers and incorporated six SR-E molecules in each lipid bilayers. These bilayer systems were simulated by long MD simulations. The analyses of trajectories are ongoing.

Abstracts

– Lipid biophysics –

P-113

Effects of MinD binding on the dynamics of a model membrane

S. Mazor[1], T. Regev[1], E. Mileykovskaya[2], W. Margolin[2], W. Dowhan[2], I. Fishov[1]

[1]Department of Life Sciences, Ben-Gurion University of the Negev, P.O.B. 653, Beer-Sheva 84105, Israel, [2]University of Texas-Houston Medical School, P.O.B. 20708, Houston, TX 77225, USA

In *E. coli* MinD, along with MinE and MinC, rapidly oscillates from one pole of the cell to the other for correct placement of the division septum. MinD has a clear preference to acidic phospholipids and, on the other hand, existence of lipid domains in bacterial membrane was recently demonstrated. We hypothesize that binding of MinD to the membrane, promoted by the ATP-induced dimerization, is further enhanced by a consequent attraction of acidic phospholipids and formation of a stable proteo-lipid domain. Furthermore, MinD dimerization and anchoring should be encouraged on preexisting anionic phospholipid domains. In the context of this hypothesis we studied changes in dynamics of a model membrane caused by MinD binding using membrane-embedded fluorescent probes as reporters. A remarkable increase in membrane viscosity and order upon MinD binding to acidic phospholipids was evident from the pyrene and DPH fluorescence changes. This viscosity increase is cooperative with regards to concentration of MinD-ATP, but not of the ADP form, supporting the dimerization. The mobility of pyrene-labeled PG indicates formation of acidic phospholipid-enriched domains in a mixed acidic-zwitterionic membrane at particular MinD/phospholipid ratios. MinD binding to the membrane can be enhanced by segregation of anionic phospholipids to fluid domains in a gel-phase zwitterionic environment and, moreover, the protein stabilizes such domains.

P-114

The influence of cholesterol on human calcitonin channel formation in planar lipid membranes

D. Meleleo[1], S. Micelli[1], V. Picciarelli[2], E. Gallucci[1]

[1]Dept. Farmaco-Biologico, [2]Dept. Interateneo di Fisica, Università degli Studi di Bari, I-70126, Bari, Italy

In aqueous solutions, human calcitonin (hCt), a 32 amino acid peptide, forms insoluble fibrils due to a hydrophobic interaction between congener molecules. In addition, hCt can interact with lipid membranes to form channels (Stipani V. et al., Bi.J. 81:3332-38,2001). It has been shown that the type of host lipid has a pronounced influence on hCt fibrillation (Micelli S. et al., Front Biosci. 11:2035-44,2006; Epand R.M. et al., Biochemistry, 22:5074-84, 1983; Wang S.S. et al., Protein Sci. 14:1419-28, 2005). Several studies have suggested that cholesterol (Ch), an integral component of eukaryotic cell membranes, promotes the transition from β-sheet to α-helix of many proteins and peptides (Ji S.R. et al., J. Biol. Chem. 277:6273-79, 2002; Micelli S. et al., B.J., 86:2231-37, 2004; Luo X.Y. et al., B.J., 92:1585-97, 2007; Padro C. et al., E.B.J. 1-36, 2003). In this study, we report data on the influence of different cholesterol concentrations on hCt incorporation and channel formation in POPC PLM. The voltage-clamp technique was used to carry out the experiments. The results indicate that single-channel activity appears when the Ch amount in the bilayer increases to 30% (molar ratio). The properties of this channel are compared with those of channels obtained in membranes of different compositions. Similar results have been observed with beta-amyloid (1-40) peptide-forming channels, suggesting that membrane cholesterol facilitates peptide incorporation. Thus, cholesterol concentration in the membrane may prove useful in controlling amyloid formation.

P-115

Local mapping of mechanical properties using suspended lipid bilayers as "nano drums"

I. Mey[1], S. Steltenkamp[2], C. Steinem[3], A. Janshoff[1]

[1]Institut f. Physik. Chemie, Johannes-Gutenberg-Universität, Jakob-Welder-Weg 11, 55128 Mainz, Germany, [2]Dept. of Chem. Engineering II, University of California, Santa Barbara, CA 93106, USA, [3]Institut f. Org. und Biomol. Chemie, Universität Göttingen, Taumannstrasse 2, 37077 Göttingen, Germany

In order to understand the elastic behaviour of living cells and their membranes complexity has to be reduced by using model systems like suspended lipid bilayers. This fairly new model system, the so called "nano drums", which bridges the gap between solid-supported and black lipid membranes, has a high long-term stability and good accessibility for surface sensitive tools like AFM. Indenting those "nano drums" with a conical AFM tip allows us to determine the elastic response of pore spanning membranes. Here, we are analysing artificial membranes consisting of DPPC, POPC, DPhPC, polymerised lipid bilayers and lipid/cholesterol mixtures to study the effects of headgroups, chain interaction and steroids on the mechanical properties. In addition to compare the experimental results to calculated predictions, we are able to calculate the elastic properties of the membrane, by solving the corresponding shape equations of continuum curvature elasticity theory. Since the measured elastic response depends in a predictable way on the system geometry (pore size, tip radius) and on material parameters (bending modulus, lateral tension), this opens the possibility to monitor local elastic properties of lipid membranes and to distinguish between the influence of headgroups, chain interaction and steroids on bending modulus and surface tension.

P-116

Ellipsometry, LSM and Z-scan FCS: interaction of antimicrobial peptides with planar lipid bilayers

A. Miszta[1], R. Machan[1], W. T. Hermens[2], M. Hof[1]

[1]J. Heyrovsky Institute of Physical Chemistry of ASCR, Prague, Czech Republic, [2]Cardiovascular Research Institute Maastricht, Maastricht, Netherlands

Antimicrobial peptides (AMPs) have received a lot of attention for their promising therapeutical applications. It has been firmly established that their interaction with cellular membranes plays a key role in the biological activity of AMPs. We used in our present study supported phospholipid bilayers as a well defined model of biological membranes and peptides magainin 2 (an example of AMPs with alfa-helical structure) and cryptdin-4 (an example of AMPs with beta-sheet structure). Combination of different complementary experimental techniques provided us with quite complex information on the manner of interaction of respective peptides with membranes. Laser scanning microscopy showed appearance of inhomogeneities in spatial distribution of lipids in the sample and the decrease of overall fluorescence intensity indicated loss of lipids from the bilayer. Ellipsometry gave us information about mass and thickness of adsorbed layer consisting of both lipids and peptides. Finally Z-scan FCS provided us with information on changes in mobility of lipids in membranes. From the results we obtained we concluded that cryptdin-4 and magainin 2 differ significantly in the manner in which they interact with biological membranes. Magainin 2 induces degradation of membranes accompanied by a significant loss of lipids and formation of small protruding lipid structures, while cryptin-4 leaves membranes confluent. It makes them more rigid (decrease in diffusion coefficient) and induces inhomogeneities in lateral distribution of lipids.

We thank MSMT CR for support via grant LC06063.

Abstracts

– Lipid biophysics –

P-117

Ellipsometry, LSM and Z-scan FCS: interaction of antimicrobial peptides with planar lipid bilayers

A. Mizsta[1], R. Machan[1], W. T. Hermens[2], M. Hof[1]

[1]J. Heyrovsky Institute of Physical Chemistry of ASCR, v.v.i, Prague, Czech Republic, [2]Cardiovascular Research Institute Maastricht, Maastricht, Netherlands

Antimicrobial peptides (AMPs) have received a lot of attention for their promising therapeutical applications. It has been firmly established that their interaction with cellular membranes plays a key role in the biological activity of AMPs. We used in our present study supported phospholipid bilayers as a well defined model of biological membranes and peptides magainin 2 (an example of AMPs with alfa-helical structure) and cryptdin-4 (an example of AMPs with beta-sheet structure). Combination of different complementary experimental techniques provided us with quite complex information on the manner of interaction of respective peptides with membranes. Laser scanning microscopy showed appearance of inhomogeneities in spatial distribution of lipids in the sample and the decrease of overall fluorescence intensity indicated loss of lipids from the bilayer. Ellipsometry gave us information about mass and thickness of adsorbed layer consisting of both lipids and peptides. Finally Z-scan FCS provided us with information on changes in mobility of lipids in membranes. From the results we obtained we concluded that cryptdin-4 and magainin 2 differ significantly in the manner in which they interact with biological membranes. Magainin 2 induces degradation of membranes accompanied by a significant loss of lipids and formation of small protruding lipid structures, while cryptin-4 leaves membranes confluent. It makes them more rigid (decrease in diffusion coefficient) and induces inhomogeneities in lateral distribution of lipids.

P-118

An optical studies of red blood cells sedimentation

J. Mutrynowska, B. Grzegorzewski

Biophysics Department, Collegium Medicum of the Nicolaus Copernicus University,Bydgoszcz, Poland

The purpose of the study was to investigate the formation of RBC deposit. Sedimentation of red blood cells (RBCs) was investigated by means of an optical method. Blood was obtained from healthy donors and measurements were performed at initial haematocrit ranged from 30 to 50%. The intensity of light transmitted through blood sample was measured as a function of time and the distance from the bottom of the container. Changes in the intensity manifest the RBC aggregation and formation of the RBC deposit. An analysis of the intensity revealed an interface between sedimenting aggregates and forming deposit of RBCs. The deposit formation curve, exemplifying the growth of the RBC deposit and the packing of the cells, was evaluated. It was shown that the duration of the phase of growth increases as haematocrit increases. An empirical formula was found to describe the formation of the RBC deposit. It was shown that parameters of the formula can be useful in the study of RBC sedimentation.

P-119

Assessing lipid raft membranes by simulation

P. S. Niemelä[1], S. Ollila[1], M. T. Hyvönen[2], M. Karttunen[3], I. Vattulainen[4]

[1]Laboratory of Physics and Helsinki Institute of Physics, Helsinki University of Technology, Finland, [2]Wihuri Research Institute, Helsinki, Finland, [3]Department of Applied Mathematics, University of Western Ontario, Canada, [4]Institute of Physics, Tampere University of Technology, Finland, and MEMPHYS-Center for Biomembrane Physics, University of Southern Denmark

Sphingomyelin (SM) molecules are, together with phosphatidylcholines (PC) and cholesterol, most important lipid components of cellular plasma membranes. The molecular structures of SM and PC are similar, but the higher hydrogen bonding capacity and the higher degree of saturation of the fatty acyl chains makes SM likely to interact with cholesterol. This interaction has been suggested to be the driving force in formation of lateral domains called rafts, which might have an important functional role through sorting and regulating the activity of membrane proteins.

We are carrying out computational work to determine properties of lipid bilayers related to SM. We have recently utilised atom scale molecular dynamics simulations to study the properties of pure SM bilayers, the molecular interactions between the suggested key lipid components in raft formation. The present contribution concentrates on comparing the results on bulk properties of raft and non-raft membranes [*PLoS Comp. Biol.* ***3*** *(2007) e34*]. The results have yielded biologically important conclusions, for example the properties of the lipid environment have been shown to be able to affect the functionality of membrane proteins.

P-120

Adaptation of human cells to environmental changes and acoustotaxis

S. Nuschele, K. Kaufmann, A. Wixforth, M. F. Schneider

University of Augsburg, Experimental Physics 1, Universitätsstraße 1, D - 86159 Augsburg, Germany

Cell migration is a well known phenomenon. Often it takes place as a response to external signals, known as chemotaxis. We investigate the effect of nanoscale sound waves on the growth behaviour of living cells (acoustotaxis). We grow different kinds of human cells like neurons on a chip in the presence of surface acoustic waves (SAW). Depending on the frequencies and magnitudes of the SAWs, cells respond with directed migration and changes in shape. An important role plays the form of SAWs because applied shear waves and Rayleigh waves carry different levels of energy in various directions into the surface area of the chip.

SAWs also function as nanopumps. In our novel microfluidic system this enables us to observe small amounts of biological material in permanent shear flow conditions.

Abstracts

– Lipid biophysics –

O-121

Model membrane phase behaviour and detergent dissolution

P. D. Olmsted
School of Physics & Astronomy, University of Leeds, U.K.

Mixed lipid membranes are fascinating both for the astonishing variety of phase behaviour they display as well as the wide potential functional features encoded in the different molecules that comprise them. Only belatedly has their importance in biological function and control been widely recognized, and now they are attracting intense interest from biologists, chemists, physicists, and even mathematicians. In this talk I will describe some recent theoretical work to understand the phase behaviour of the canonical "lipid raft" mixture, as well as experimental studies of detergent dissolution of membranes as a function of their composition.

P-122

Characterizing drug delivery systems using fluorescence solvent relaxation technique

A. Olzynska, P. Jurkiewicz, J. Sykora, M. Hof
J. H. Institute of Physical Chemistry, Prague, Czech Republic

Fluorescence solvent relaxation technique (SR) enables to study hydration and dynamics of the lipid membranes.[1] Relaxation process refers, here, to dynamic reorganization of water hydrating the membrane as a response to a rapid change in the fluorophore dipole moment upon excitation. The overall Stokes shift correlates with the probe environment polarity and thus reflects the degree of membrane hydration. The kinetics of the time-resolved Stokes shift reports on mobility of the probe environment (mobility of hydrated lipids in case of membranes).[1,2]

We examine surface properties of Transferosomes® (highly deformable lipid vesicles designed for transdermal drug delivery), important for the drug loading efficiency. Headgroup hydration and mobility of two types of mixed lipid vesicles, containing nonionic surfactants; straight chain Brij 98 and polysorbat Tween 80, are investigated. The interactions of the systems with protein Interferon alfa-2b are also addressed.[3]

We also apply SR technique to characterize positively charged lipid membranes (important for the gene therapy). We compare binary lipid mixtures consist of a cationic lipid (DOTAP[2] or DMTAP) and a neutral helper lipid (DOPC, DMPC or DOPE). Thanks to the sensitivity of the method we were able to characterize those systems at the molecular level. The obtained results are in agreement with the molecular dynamics simulations performed lately.

References: [1] *J. Fluorescence* 15 (2005) 883. [2] *Langmuir* 22 (2006) 8741. [3] *Biochim. Biophys. Acta* 1768 (2007) 1050.

P-123

A quantitative coarse-grain model for lipid bilayers

M. Orsi[1], W. Sanderson[2], J. W. Essex[1]
[1]School of Chemistry, University of Southampton, Highfield, Southampton, U.K., [2]Johnson & Johnson PRD - Janssen Pharmaceutica NV, Turnhoutseweg 30, Beerse - 2340, Belgium

A simplified particle-based computer model for hydrated phospholipid bilayers has been developed and applied to quantitatively predict all major physical features of fluid-phase biomembranes. Each lipid molecule, which in reality comprises about one hundred atoms, is represented as a collection of ten coarse-grain particles. Hydrocarbon tails are modeled using the anisotropic Gay-Berne potential. Water is described by the soft sticky dipole model. Electrostatics are incorporated via charges and dipoles. Simulations are conducted by rigid-body molecular dynamics. Our technique proves two orders of magnitude less demanding of computational resources than traditional atomic-level methodology. The model is parameterized to reproduce the experimental area and volume per lipid, order parameters, and the self-assembly process. Self-assembled bilayers quantitatively reproduce experimental observables such as electron density, compressibility moduli, dipole potential, lipid diffusion and water permeability. The lateral pressure profile has also been calculated, along with the elastic curvature constants of the Helfrich expression for the membrane bending energy: results are consistent with experimental estimates and atomic-level simulation data. Our model can provide quantitative insights, at molecular resolution, into the physical properties at the basis of crucial biological processes, including phase transition, fusion, growth, transport and signaling.

P-124

NMR studies of binary and ternary systems of phosphatidylcholines, sphingomyelins and sterols.

G. Orädd[1], G. Lindblom[1], K. Halling[2], T. K. M. Nyholm[2], P. J. Slotte[2]
[1]Dept. of Chemistry, Umeå University, SE-901 87, Umeå, Sweden, [2]Dept. of Biochem. and Pharmacy, Åbo Akademi University, 205 20 Turku, Finland

Lipid lateral segregation in biomimicking model membranes has recently become a topic of great interest due to the biological coupling to the concept of functional rafts. In the studies of liquid-liquid phase coexistence it is found that lateral phase separation into liquid ordered and liquid disordered phases takes place in ternary systems consisting of one unsaturated and one saturated lipid, together with a sterol. The phase behavior is critically dependent on the components with respect to chain length, degree of unsaturation and sterol structure and the driving force of the phase separation is closely related to the ordering effect of the sterol on the lipid chains.

This study presents NMR data for binary and ternary systems composed of mixtures of sphingomyelins, phosphatidylcholines and cholesterol. Order parameters were obtained from perdeuterated chains in the saturated lipid and diffusion data of both the saturated and unsaturated lipids were measured with the pulsed field gradient NMR method. Data were analyzed with regard to phase separation into liquid and solid phases.

Abstracts

– Lipid biophysics –

P-125

On the propensity of phosphatidylglycerols to form interdigitated phases

G. Pabst[1], S. Danner[1], A. Hickel[1], V. A. Raghunathan[2]
[1]Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, Schmiedlstr. 6, 8042 Graz, Austria, [2]Raman Research Institute, Bangalore 560 080, India

Using calorimetry, dilatometry, as well as x-ray diffraction we have determined the phase behavior of disaturated phosphatidylglycerols (PGs) of chain lengths n_{CH2} = 14 - 18 at pH 7.4 and in the presence of 130 mM NaCl. We find that PGs with n_{CH2} = 14 and 16 show thermotropic behavior similar to that of phosphatidylcholines (PCs). However, the lateral area/lipid obtained in the gel phase is smaller than that reported for PCs despite the expected larger effective headgroup size. This can be explained by a tilt of the PG headgroup out of the bilayer plane and we provide experimental evidence for a headgroup tilt transition. For distearoyl PG, we further find that the "usual" gel phase coexists with an interdigitated phase, which exhibits a transition from an orthorhombic into a hexagonal chain packing as a function of temperature. The total amount of the interdigitated phase depends significantly on the temperature, but is found to be largely independent of temperature equilibration time and different sample preparation protocols. Thus, the development of the interdigitated phase appears to be kinetically trapped. Using the frog-skin peptide PGLa as an example we further demonstrate the relevance of these findings to interaction studies with antimicrobial peptides, which appear to further promote the tendency of PGs to interdigitate.

This work has been supported by the Austrian Science Fund FWF (Project No. P17112-B10).

P-126

Study of the interaction of GBV-C/HGV derived peptides with liposomes by fluorescence methods

C. Padró[1], J. Prat[1], M. A. Alsina[1], I. Haro[2], M. Pujol[1], M. A. Busquets[1]
[1]Department of Physical Chemistry. IN2UB. University of Barcelona. Spain, [2]Department of Peptide and Protein Chemistry. IIQAB-CSIC. Barcelona. Spain

With a view toward possible new insights into viral fusion mechanisms, we have investigated by various fluorescence techniques the interaction of several sequences corresponding to the HGV/GBV-C virus with Large unilamellar vesicles (LUVs) of DMPC alone or with a 50% mol of DMPG as model membranes. The potential of the peptides to induce membrane fusion was analysed by lipid mixing fusion assays based on fluorescence resonance energy transfer with NPB-PE (acceptor) and Rho-PE (donor). On another hand, peptide incorporation and location in the phospholipid bilayer was investigated by fluorescence anisotropy with LUVs labeled with DPH (located at the inner part of the bilayer) or TMA/DPH (located at the surface with the polar head in contact with the phospholipid polar head and the hydrophobic tail in contact with the phospholipid acyl chains). Finally, release contents from liposomes composed of POPG was performed with the ANTS/DPX vesicle-fusion assay. Peptides were able to change the bilayer properties in a different extent depending on the lipid composition. In a general trend, sequences having a serine, arginine or glycine as terminal residue showed the highest interaction with liposomes.

P-127

Spectroscopic and calorimetric studies of HSA interacting with PEG:2000-DPPE/DPPC membranes

M. Pantusa, L. Sportelli, R. Bartucci
Physics Department, University of Calabria, Italy

The molecular interactions at the lipid-protein interface have a great biophysical interest because of the development of protein-resistant surface coatings obtained by inclusion of polymer-lipids in vesicle membranes, which are used as long circulating drug carriers.

We have studied the interaction of the most abundant plasma protein, human serum albumin (HSA), with membranes composed of common diacyl lipids of dipalmitoylphosphatidylcholine (DPPC) and sub-micellar amounts of the polymer-lipid dipalmitoylphosphatidylethanolamine-PEG:2000 (PEG:2000-DPPE). The three domains of HSA have been investigated with different techniques: domain I by electron spin resonance labelling the unique free sulfhydryl group at Cys34 with a maleimido spin-label; domain II by intrinsic fluorescence of the single Trp214, and domain III by the extrinsic fluorescence of *p*-nitrophenyl anthranilate conjugated with Tyr411. Differential scanning calorimetry was also used to get insight into molecular properties of dispersions of HSA with or without polymer-grafted membranes.

The results evidence that the HSA domains possess different properties and are differently affected by the interaction with DPPC. The protein adsorbs to the surface of DPPC membranes and undergoes an up-shift of the thermal unfolding temperature and a decrease of the transition enthalpy. The primary protein adsorption is strongly reduced at low mushroom content of the polymer-lipids. The secondary adsorption at the polymer brush restricts the protein dynamics and increases the thermodynamic stability of HSA.

P-128

Studies on interaction of bioactive compounds with chromatic giant vesicles

A. Pevzner, S. Kolusheva, R. Jelinek
Department of Chemistry, Ben-Gurion University of the Negev, 84105 Beer-Sheva, Israel

A new colorimetric/fluorescent assay has been developed for investigation of membrane perturbation and interaction modes of bioactive compounds, such as antimicrobial peptides, pharmaceutical substances and viruses with lipid constituents of the membrane. This technique is based on application of giant vesicles composed of different phospholipids embedded in a matrix of chromatic polydiacetylene moieties. The meso-scaled size of the vesicles (5-20 μm) makes them very attractive practical tool for visualization of membrane processes using conventional optical systems, including Fluorescence and Phase Contrast Microscopy.

Abstracts

– Lipid biophysics –

P-129

Changes of erythrocyte shape and membrane fluidity induced by organotins

S. Przestalski, D. Bonarska-Kujawa, H. Kleszczyńska
Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Norwida 25,50-375 Wrocław, Poland

Organic compounds of tin present in the environment are toxic to living organisms. As for now, the mechanism of their toxic action on the cell and molecular levels has not yet been finally explained. Therefore, it was studied the effect of the above mentioned compounds on the erythrocyte membrane, assumed to be a model of the biological membrane. The results obtained show that the compounds triphenyltin and tributyltin chlorides, diphenyltin and dibutyltin dichlorides, alter the shape of erythrocytes and affect their membrane fluidity. In particular, using an optic microscope it was observed that under the influence of the substances mentioned erythrocytes change their shape, becoming mainly echinocytes. The changes depend on the kind of compounds and indicate that their concentrations in either of the membrane lipid monolayers differ. Fluidity of erythrocyte membranes was investigated at various depths of the lipid bilayer using properly chosen fluorescent probes: laurdan, TMA-DPH and DPH. The results obtained indicate that organotins incorporate into the membrane at various depths, whereby they affect the erythrocyte shape and membrane fluidity to various degrees. This, in turn, testifies that the study of the erythrocytes shape may explain some aspects of interaction mechanism.
This work was sponsored by the Polish State Committee of Scientific Research, grant no. 2 P04G 089 27.

P-130

Mechanisms involved in surfactant inhibition by CRP: The protective role of SP-A

A. Saenz, C. Casals
Dep of Biochem and Mol Biol, Complutense University of Madrid, Spain

pulmonary surfactant is a lipid-protein complex that covers the alveolar surface, prevents alveolar collapse, and contributes to lung defense. It is sensitive to functional inhibition by plasma proteins, which invade the alveolar space during acute lung injury. C-reactive protein (CRP) is a plasma protein that notably increases in bronchoalveolar lavage following tissue injury, infection, or inflammation. CRP may interact with surfactant phospholipids in the alveolar space, resulting in impairment of surfactant activity. Surfactant protein A (SP-A), the most abundant lipoprotein of pulmonary surfactant, is involved in multiple biological functions, including improvement of biophysical activity of pulmonary surfactant and host defense. Besides, SP-A has the ability to prevent surfactant inhibition by serum proteins. The aim of this study was to investigate the mechanisms involved in surfactant inhibition by CRP and to determine whether SP-A reverses this inhibition. The effects of CRP and CRP/SP-A on the physical properties and surface activity of two different surfactants (natural and synthetic) were assessed by steady-state emission anisotropy of 1,6-diphenyl-1,3,5-hexatriene, differential scanning calorimetry, and interfacial adsorption studies. The results indicated that CRP inhibited surface adsorption of lung surfactant by increasing membrane fluidity of either natural or synthetic surfactant. The presence of SP-A blocked all CRP effects on surfactant membranes as well as the inhibitory effect of CRP on surface adsorption. The results suggest a potential interaction between SP-A and CRP, which might prevent surfactant inactivation by this serum protein.

P-131

Phospholipid binding properties of the Brichos domain-containing C-terminal part of proSP-C

A. Saenz[1], H. Johansson[2], J. Johansson[2], C. Casals[1]
[1]Dept Biochem. & Mol. Biol, Complutense University of Madrid, 28040 Madrid, Spain, [2]Dept. Anat. Physiol. & Biochem., Swedish University of Agricult. Sci., 751 23 Uppsala, Sweden

The lung surfactant peptide SP-C contains a long α-helix composed mainly of a poly-Val stretch, which is prone to convert into β-sheet aggregates and amyloid-like fibrils. SP-C corresponds to the transmembrane part of its precursor proSP-C, which is an integral transmembrane protein in type II orientation, meaning that the C-terminal part (CTproSP-C) is located in the ER lumen. The Brichos domain-containing CTproSP-C binds SP-C in β-sheet conformation and might work as a scavenger to prevent aggregation of the non-helical poly-Val segment of proSP-C. We hypothesize that CTprosp-c interacts with the membrane surface, facilitating the recognition of the poly-Val stretch in β-sheet conformation. In this study we investigate CTproSP-C/membrane interaction using a variety of techniques, including intrinsic and extrinsic fluorescence, CD, surface balance, and DSC. We found that CTproSP-C interacts with the membrane surface without penetrating deeply into the hydrophobic region. CTproSP-C binds to both neutral and negatively charged membranes (K_d= 6±0.8 μM). Phospholipid binding to CTproSP-C induces a conformational change in the protein so that membrane-bound CTproSP-C is less structurally ordered than the unbound protein. Interaction of CTproSP-C with the membrane produces increased lipid packing. We suggest that CTproSP-C may function not only to prevent aggregation of the poly-Val segment of SP-C, but also to reduce the "free volume" available in the membrane for α-helix to β-sheet conformational changes in proSP-C.

P-132

Transport and effects of chlorpromazine on lipid bilayers

E. Salonen[1], K. Heikkilä[2], I. Vattulainen[2]
[1]Laboratory of Physics and Helsinki Institute of Physics, Helsinki University of Technology, Finland, [2]Institute of Physics, Tampere University of Technology, Finland

Chlorpromazine (CPZ), a drug of the phenothiazine family, has been used as an anti-psychotic since the early 1950s. Although it is the oldest drug of its kind, and its physiological effects are well known, the modes of action of CPZ at the molecular level are not well understood. This further illustrates the general lack of biochemical understanding of the class of diseases collectively known as schizophrenia [1].
Before reaching their final site of action in the central nervous system (CNS), anti-psychotics have to permeate through a number of lipid-dominated barriers (e.g., the blood-brain barrier). It is not known how CPZ molecules affect the properties of these barriers, or the plasma membranes of neurons in the CNS.
Using molecular dynamics simulations, we have studied how CPZ molecules partition into and permeate model phospholipid membranes. Both the unprotonated and protonated forms of CPZ are featured in the modeling. The modeling provides a comprehensive picture on the structural properties and dynamics of phospholipid-CPZ systems at the molecular scale. The results of the modeling are compared to experimental studies of similar model systems [2,3].
[1] D. M. Perrine, The Chemistry of Mind-Altering Drugs - History, Pharmacology, and Cultural Context (American Chemical Society, Washington, DC, 1996).
[2] A. Jutila *et al*., Chem. Phys. Lipids 112 (2001) 151.
[3] A. V. Agasosler *et al*., Biochem. Pharmacol. 61 (2001) 817.

Abstracts

– Lipid biophysics –

P-133

Biological activity of some novel N-oxides

J. Sarapuk[1], H. Kleszczyńska[1], K. Bielecki[2], D. Bonarska-Kujawa[1]

[1]Dpt. of Physics and Biophysics, University of Environment and Life Sciences, Wrocław, Poland, [2]Dpt. of Plant Nutrition, University of Environment and Life Sciences, Wrocław, Poland

There is a continuous search for new compounds that can be applied biologically in various character. A possible application of two series (chlorides and bromides) of newly synthesized *N*-oxides of tertiary amines (NTA) was searched for. Particular compounds in each series differed in the alkyl chain length.

Biological efficiencies of NTA were determined by studying their influence on the growth of cucumber, chlorophyll content and potassium leakage from cucumber. The interaction of NTA with model membranes (erythrocytes) was also studied.

The potential antioxidative efficiency of NTAs was studied by determining the degree of the protection of ghost membranes against UV induced peroxidation and in radical chromogen experiments.

It was found that the influence of both series on the growth of cucumber hypocotyls, chlorophyll content and potassium efflux increased with the alkyl chain length, bromide series being more effective. The same effects were found in hemolytic experiments.

The antioxidative protection of ghost membranes was quite good, and better for chloride compounds perhaps due to their weaker interaction with membranes. However, chromogen tests did not confirmed that the N-oxides studied have good antioxidative efficiencies.

The work was sponsored by the Polish Research Committee (KBN), grant no. 2 P04G 088 27.

P-134

Determination of oxidative stress and protein oxidation in chronic obstructive pulmonary disease (COPD)

D. Saribal[1], M. C. Akyolcu[1], B. Aydemir[1], A. Kacakci[1], V. Yilmaz[2]

[1]Istanbul University, Cerrahpasa Medical Faculty, Department of Biophysics, Istanbul, Turkey, [2]Yedikule Respiratory Diseases and Thoraxic Surgery Education and Research Hospital, Istanbul, Turkey

Most frequently observed symptoms of COPD are obstruction of inspiration and expiration. Increased concentration of free radicals and ROS is observed against inflammatory reaction depending to the air way obstruction due to COPD. The monovalent reduction of O_2 gives rise to highly reactive O_2 intermediates, such as superoxide radical ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$), as well as to singlet O_2. By reacting of the oxidants with proteins, lipids and nucleic acids lead to cell dysfunction or cell death. There are, however, several lines of antioxidant defenses, which can be enzymatic (superoxide dismutase, catalase, glutathione peroxidase) or non-enzymatic (glutathione). Present study was performed to determine the oxidative stress and protein oxidation in chronic obstructive pulmonary disease. While 25 male COPD patients (during acute attack) were taken as a patient group another healthy 25 male taken as control group. Statistical evaluations were realized according to student's t test.

As a lipid peroxidation product MDA was found to be statistically higher in plasma and erythrocytes but GSH and Catalase enzyme activity lower in erythrocytes of patient group than that of controls. ($p < 0, 001$). Protein oxidation in serum of patients was higher than controls ($p<0,01$). As a result it may be said that COPD patients expose to lipid peroxidation and protein oxidation.

P-135

Lipid phase transition temperature modifications by microwave irradiation of model membranes

T. Savopol[1], E. Kovacs[1], N. Iacob[2], D. Martin[2], L. Bajenaru[1], S. Miclaus[3]

[1]Biophysics and Cell Biotechnology Dept., Carol Davila Medical University, P.O. Box 35-43, 050461 Bucharest, Romania, [2]National Institute for Lasers, Plasma and Radiation Physics, Bucharest-Magurele, Romania, [3]Land Forces Academy, Sibiu, Romania

We present a study on the effects of microwaves of different powers on the fluidity of and polarizability of model membranes (dimiristoyl phosphatidyl choline liposomes labeled with TMA-DPH or Laurdan respectively). We monitored the fluorescence anisotropy of TMA-DPH and the generalized polarization of Laurdan during the heating by microwave irradiation, which allowed determining the transition temperature of the membrane under these conditions. We compared these values with transition temperatures determined by heating the samples using thermal contact (with a computer programmed thermostat, which allowed us to keep the same temperature – time profile as in the case of irradiated samples).

We observed a rising of the transition temperature by a few degrees centigrade, depending on the applied microwaves power.

The results are interpreted in terms of membrane destabilization by water penetration in the lipidic bilayer above the critical temeperature, which seems to be affected by the presence of the electromagnetic field.

P-136

Measuring the elastic properties of vesicle membranes by tether manipulation

L. Schmid, A. Wixforth, T. Franke

Experimental Physics I, University of Augsburg, Germany

We investigate the elastic and viscous properties of giant unilamellar vesicles (GUVs).

We use paramagnetic particles trapped in phospholipid giant vesicles to manipulate the vesicles by applying an external magnetic field. By applying a strong enough force, membrane tethers of lengths of several hundred μm can be can be pulled out of the vesicle. The piconewton pulling force is measured. With theoretical modeling it is possible to deduce bending stiffness and tension of the vesicle membrane.

In another set of experiments, pearling instabilities of membrane tethers are observed. The pearls exhibit one-dimensional diffusion movement along the tether axis. The diffusion constant is shown to depend on inter-membrane dissipation, leading to a new way to measure membrane viscosity. Corresponding viscosity measurements were performed.

Abstracts

– Lipid biophysics –

P-137

Solvent free pore-suspending membranes on nano-porous materials

E. K. Schmitt, C. Steinem
Institute of Organic and Biomolecular Chemistry, Tammannstraße 2, 37077 Göttingen, Germany

Lipid bilayers spanning nanometer-sized pores of porous substrates combine the stability of solid supported membranes with the advantages of freestanding lipid bilayers. In contrast to nano-BLMs, we aim to generate membranes, which are nominally solvent free, which is a prerequisite for the functionality of some transmembrane proteins. The strategy followed in the work presented here was to functionalize the porous substrate with a monolayer of CPEO3, an amphiphilic spacer molecule containing a cholesterol residue attached to a hydrophilic anchor built up of three ethoxy units (PEO3). A thiol function allowed for the chemisorption to gold coated porous substrates of alumina (60 nm pores) or silicon (800 nm pores). A complete lipid bilayer was established by spreading vesicles covering both, the solid part of the substrate and the pores. Minimum amounts of n-decane were applied to the sealing ring region to minimize leak currents.The formation process, the electrical parameters as well as the long-term stability were investigated by electrical impedance spectroscopy (EIS). The data indicate the generation of a highly insulating layer with a total resistance of $>10^6$ Ω across the porous substrate. The functionality of the pore-spanning membranes was verified by the insertion of the peptide alamethicin and the reversible reconstitution of the protein OmpF, followed by EIS. Furthermore, we applied liposomes containing 0.1 mol% of the fluorescent marker lipid Texas Red DHPE to CPEO3 functionalized porous silicon substrates to visualize the pore-spanning bilayers by fluorescence microscopy.

P-138

Does biology make use of membrane phase transitions ?

M. F. Schneider
University of Augsburg, Experimental Physics I, Biological Physics Group, Universitätstr. 1, D-86159 Augsburg, Germany

The mechanical properties of lipid membranes enable vesicles to go through morphological transitions, which may play an important role during endo or exocytosis. Coats of proteins are believed to alter the mechanical properties of the hybrid shell. However, the effect of the membrane – protein interaction on the mechanical properties itself was not considered until now.
Here we study the possible role of phase transition triggered membrane trafficking. We were able to induce fusion, budding adhesion and vesicle transport over the membrane by lipid phase transitions. Knowing, that all proteins involved in membrane budding contain a hydrophobic anchor, which is able to shift the membrane phase transition, we discuss protein adsorption as a possible mechanism to induce phase transition and therefore budding and fusion of lipid membranes.
Furthermore the effects of lipid chain melting on current fluctuations have been studied. Current events similar in time scale ($\sim$ 1-100ms) and amplitude ($\sim$ 10 pA) to those known from ion channels containing membranes are observed. Concerning the membranes phase state, we report a 100fold increase in event time in the lipid phase transition regime when compared with the fluid phase.
Finally we would like to ask whether there are any known morphological changes in biomembranes (e.g. during membrane trafficking) or current fluctuations, which are in principle not observable in protein free membranes too.

P-139

Gel phase disruption in sphingomyelin-cholesterol systems

K. P. Shaw, J. M. Seddon, R. V. Law
Chemistry Department, Imperial College, London, U.K.

There is currently great interest in SM/Chol phospholipid bilayers due to their relevance to lipid rafts. At sufficiently high concentrations, Chol acts to disrupt the gel phase (L_β) and order the fluid phase (L_α) of SM, leading to the formation of the 'intermediate' liquid ordered (L_o) phase. This phase retains characteristics of both the L_β and L_α phases and has been strongly associated with lipid rafts.
Using a combination of x-ray diffraction and ^{31}P solid-state NMR, we have investigated the disruption of the L_β phase of SM at low Chol concentrations. The gel phase exhibits regular in-plane hexagonal packing of the phospholipids hydrocarbon chains. From our x-ray scattering data we have observed a broadening of the wide angle diffraction peak with increasing Chol content, and we interpret this as a disruption of this packing in the gel phase. The ^{31}P NMR data provides further evidence for this disruption and also shows increased molecular motion in SM.
In addition, we have also looked at the effect of increasing Chol concentration on the ^{31}P MAS (magic angle spinning) linewidth of SM. For SM we see a sharp increase in linewidth prior to formation of the fluid phase. This increase is gradually reduced by incorporation of Chol until, at 15mol%, there is a continuous decrease in linewidth. We have investigated this phenomenon further by looking at other phospholipid systems.

O-140

A novel mechanism for drug transport through membranes

G. C. Shearman[1], S. C. Sebai[1], X. Mulet[1], M. Baciu[1], O. Ces[1], J. A. Clarke[1], R. V. Law[1], R. H. Templer[1], C. Plisson[2], C. A. Parker[2], A. D. Gee[2]
[1]Department of Chemistry, Imperial College London, London SW7 2AZ, U.K., [2]GSK Clinical Imaging Centre, Hammersmith Hospital, London W12 0NN, U.K.

The two recognised models for the transportation of drugs across cell membranes are passive diffusion and active transport. We hypothesise that a third process exists for the translocation of cationic amphiphilic drugs (CADs): the chemically activated degradation of the phospholipid matrix comprising cell membranes. This was initially seen from the action of two dopamine D_2 antagonists on model membranes. The chemically activated degradation of the lipid bilayer can be described in a few steps. First, the CAD binds to the bilayer, which enables the CAD to catalyse the acid hydrolysis of phospholipid ester linkages. The membraneous fragments generated then transport the CAD to another membrane region, and the process is then repeated. Evidence for this comes from the rapid generation of monooleoylphosphatidylcholine and oleic acid upon the addition of CADs to dioleoylphosphatidylcholine in excess buffer. The degradation products were confirmed directly by normal phase HPLC, and the generation of isotropic material over time, such as vesicles, has been observed using solid state NMR. We have also shown that CADs have no effect on synthetic ether-linked lipids, corroborating our hypothesis. Visualisation of this degradative process by fluorescence imaging has shown that this happens on a pharmacological timescale, and kinetic studies have yielded the rates of hydrolysis for a selection of different CADs.

Abstracts

– Lipid biophysics –

P-141

Detection of bacteria by a new color and fluorescence sensor system

L. Silbert[1], I. Ben-Shlosh[1], S. Kolusheva[2], R. Jelinek[1]

[1]Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel, [2]ILSE KATZ Center for Meso and Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Direct detection and identification of bacteria is an important approach that can lead to important applications in several areas of microbiology, including public health, quality of environment, water and food and bio-terror prevention.
Our goal in this research is to develop simple systems for chromatic detection of bacteria in very short times.
The detection system is based on interaction of membrane-active compounds secreted by bacteria with agar-embedded nanoparticles (the nanoparticles containing phospholipids and the chromatic polymer polydiacetylene (PDA)).
The PDA undergoes dramatic visible blue-red transformations together with an intense fluorescence emission, induced by molecules released by bacteria.
When bacterial secretion binds to the biosensor surface, the backbone chain of the PDA is disrupted and the sensor changes its color from blue to red.
The chromatic technology is basic, simple and rapid, does not require identification of specific bacterial recognition elements, and can be applied for detection of both Gram-negative and Gram-positive bacteria.

P-142

Electrostatic potentials created by membrane fragments with Na,K-ATPase adsorbed on lipid membranes

V. S. Sokolov[1], E. A. Sokolenko[1], D. V. Filinsky[1], Y. A. Ermakov[1], O. D. Lopina[2], H.-J. Apell[3]

[1]Frumkin Institute of Physical Chemistry and Electrochemistry, RAS, Moscow, Russia, [2]Biological Department , Moscow State University, Moscow, Russia, [3]Biological Department, University of Konstanz, Konstanz, Germany

The electrostatic potentials created by adsorption of membrane fragments (MF) containing Na^+,K^+-ATPase on bilayer lipid membrane (BLM) were measured by different methods: second harmonic capacitive current compensation as well as change of the BLM conductance induced by nonactin or pentachlorophenol. These potentials can be generated by two different processes. The first is adsorption of single charged molecules on the BLM which can be either phospholipids dissolved in water, or traces of detergent, sodium dodecyl sulfate. The second is adsorption of charged MF leading to the generation of an electric field inside the BLM in the contact region with MF if the distance between BLM and MF is comparable to the thickness of the diffuse electric double layer. The perfusion of the cell decreased the potential to the limiting value (about 10 mV) due to washing the charged molecules from the surface of BLM. This value is assumed to be caused by irreversible adsorption of MFs. The surface charge of MFs measured by the method of electrophoretic mobility is about -10 mC/m^2. Liposomes formed from the lipids extracted from MF could also adsorb on the BLM leading to a rise of the electrostatic potential. These results allow the evaluation of the electrostatic potential in the contact region of MF and BLM which is about tens of mV.

P-143

Partition and location of enrofloxacin in unilamelar liposomes: a fluorescence spectroscopic study

I. Sousa, P. Gameiro

Requimte, Departamento de Química, Faculdade de Ciências, 4169-007 Porto, Portugal

The fluoroquinolones are a series of synthetic antibacterial agents that are used in the treatment of a variety of bacterial infections. Enrofloxacin was the first fluoroquinolone introduced into veterinary medicine. Fluoroquinolones use a transmembranar porin to pass through the outer-membrane of the gram negative bacteria, but the exact mechanism and the previously interaction between these drugs and the lipid composition of the outer-membrane, remains unknown. Insights about this mechanism can be obtained from the study of their partition and location in membrane models, such as liposomes, since they can mimic the chemical and structural anisotropic environment of cell membranes but lack the transport machinery. In this work, the partition and the location of enrofloxacin in DMPC and DMPG lipid bilayers has been studied. The application of fluorescence spectroscopy enabled the determination of the partition coefficients of this fluoroquinolone and quenching studies allowed the inference of its location in the liposomes. In view of these results, the mechanism by which enrofloxacin permeates through the phospholipids bilayer must include an electrostatic interaction at the interface region and this association must be the first step that governs its mechanism of interaction with bacterial natural membranes. This behavior is compared with that observed for other fluoroquinolones.

Acknowledgements Partial financial support for this work was provided by "Fundação para a Ciência e Tecnologia" (FCT, Lisboa) through Project POCI/SAU-FCF/56003/2004.

P-144

Development of supported bilayer technologies for the study of drug-membrane interactions

C. E. Stanley[1], O. Ces[1], A. J. de Mello[1], A. D. Gee[2]

[1]Imperial College, London, U.K., [2]GSK Clinical Imaging Centre, London, U.K.

Within the last year, Baciu *et al*. [1] have discovered what is believed to constitute a new mechanism of drug translocation between model membranes. In contrast to previous models, this mechanism proposes a degradative component; cationic amphiphilic drugs (CADs) have been shown to *chemically* degrade phospholipid membranes via acid-catalysed ester hydrolysis, to form water-soluble micelles containing the original CAD molecule. This phenomenon has been investigated using several techniques, primarily: small angle X-ray diffraction (SAXS), nuclear magnetic resonance spectroscopy (NMR) and fluorescence microscopy (FM).
More recently, supported lipid bilayer platforms are now being used to study this mechanism as they allow us to overcome many of the problems associated with vesicular assays. It is therefore proposed that SLB platforms should remove any ambiguity associated with such arrays and act as an efficient, standardised and reproducible means to study the aforementioned degradation mechanism.

[1] Baciu *et al*, *Phil. Trans. Soc. A.,* 2006, **364**, 2597-2614

Abstracts

– Lipid biophysics –

P-145

Change in lipid peroxidation and antioxidant enzymes activity in patients after cryotherapy

A. Staron[1], G. Makosa[2], P. Duchnowicz[1], M. Koter-Michalak[1]
[1]Department of Environment Pollution Biophysics, University of Lodz, 12/16 Banacha St., 90–237, Lodz, Poland, [2]Department of Rehabilitation, Hospital in Tuszyn, 5 Szpitalna Str., Tuszyn, Poland

Cryotherapy is used in cooperation with pharmacological treatment, rehabilitation and biologic restitution. Therapy is based on applying on body surface cryonical temperatures (from –160°C to –100°C, 3 min) which evokes physiological reactions. The therapy course includes 8–10 treatments, one per day. Patients spend 3 minutes in cryogenical chamber and after that they are subjected to intensive kinesitheraphy.
We determined the effect of cryotherapy on change in lipid peroxidation (TBARs) and antioxidant enzymes activity: catalase (CAT), peroxidase (GPx), superoxide dismutase (SOD) in red blood cells of patients with different diseases (spin and joints degenerative disease, rheumatoid arthrosis, multiple sclerosis).
Changes in these factors were measured in 30 patients. Blood samples were taken before cryotherapy treatment and after 8 days of treatment. All experiments were carried out in the day after sample taking.
Statistically significant decrease in lipid peroxidation and change in antioxidant enzymes (CAT, GPx, SOD) activity were observed.

P-146

Interaction of HIV-1 Nef protein with artificial membrane systems

R. Szilluweit[1], O. Fackler[2], C. Steinem[1]
[1]Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, University of Goettingen, 37077 Goettingen, Germany, [2]Department of Virology, University of Heidelberg, 69120 Heidelberg, Germany

Nef plays an important role in the pathogenesis of HIV and belongs to the so-called auxiliary proteins. The protein is composed of 206 amino acids with a molecular mass of 27 kDa. Its N-terminus is linked to a myristoyl moiety, which is required for the association with cellular membranes. The sequence of the first 25 N terminal amino acids of Nef is very similar to the sequence of the bee venom melittin, which is a membrane-active peptide. This similarity is also reflected in the molecular structure of the two molecules, although the N-terminal helix of melittin is more defined. The major aim of this study is to analyze the interaction of full length Nef with lipid membranes and investigate the influence of the myristoylation. To start with, membranes composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) immobilized on silicon dioxide were used. The impact of full length non-myristoylated Nef (mutant Nef G2A) on POPC bilayers was investigated by fluorescence and atomic force microscopy. Furthermore, we investigated the membrane protein interaction by means of ellipsometry, which enables us to monitor changes in the membrane/film thickness induced by the protein. As a result, the protein appears to be capable of disrupting the membrane similar to what has been observed for the action of melittin on POPC bilayers. A similar result was first reported in 1994 by Curtain et al., who found that full-length, non-myristoylated Nef has membrane perturbing capabilities.

P-147

Single GUV Method Reveals the Pore Formation of Magainin 2 in Lipid Membranes

Y. Tamba, H. Ariyama, M. Yamazaki
Grad. Sch. Sci. Tech, Shizuoka University, Japan

Recently we have proposed a novel method, the single GUV method. In this method we observe and measure physical properties of single giant unilamellar vesicles (GUVs) with a diameter of ≥ 10 μm, and analyze these results over many GUVs statistically, which will provide much new information that cannot be obtained by the conventional LUV suspension method. In this report, we show the application the single GUV method to the investigation of interaction of magainin 2, an antimicrobial peptide, with lipid membranes. We found that low concentrations ($3-10$ μM) of magainin 2 induced rapid leakage of a fluorescent probe, calcein, from the inside of single 50%DOPG/50%DOPC-GUVs without disruption of the GUVs, indicating that magainin 2 formed pores in the membrane [1]. The rapid leakage of the fluorescent probe from a GUV started stochastically, and once it began, the complete leakage occurred rapidly within about 1 min. This result indicates that the pore formation is the rate-determining step, rather than the leakage through the pore. Using the single GUV method, we succeeded in determining the rate constant of the pore formation [1]. We also investigated dependence of the rate constant on the surface charge density of the membrane and on magainin 2 concentration in a buffer, and the analysis of these results indicate that the concentration of magainin 2 bound with the membrane is a key factor for the pore formation. Based on these results, for the magainin 2-induced pore formation, we have proposed the model of the two-state transition from the binding state of magainin 2 to the external monolayer membrane to the pore state in the membrane. [1] Biochemistry, 44, 15823, 2005

P-148

Single GUV method reveals interaction of (-)-Epigallocatechin gallate with lipid membranes

Y. Tamba, S. Ohba, H. Yoshioka, M. Yamazaki
IJRC and Grad. Sch. Sci. Tech., Shizuoka University, Japan

Tea catechins**,** which are the main components of green tea extracts, are thought to have antibacterial activity. Several studies indicate that lipid membranes are one of the targets of the antibacterial activity of catechins. Studies using a suspension of large unilamellar vesicles (LUVs) indicate that catechin causes gradual leakage of internal contents from LUVs. However, the detailed characteristics of the interaction of catechins with lipid membranes remain unclear. In this report, we investigated the interaction of (-)-epigallocatechin gallate (EGCg), a major catechin in tea extract, with single giant unilamellar vesicles (GUVs) of egg phosphatidylcholine (egg PC) in a physiological ion concentration, using the single GUV method [1].Low concentrations of EGCg at and above 30 μM induced rapid leakage of a fluorescent probe, calcein, from the inside of single egg PC-GUVs; after the leakage, the GUVs changed into small lumps of lipid membranes. We found the detailed process of the EGCg-induced burst of GUVs, the decrease in their diameter, and their transformation into small lumps. These results indicate that the leakage of calcein occurred as a result of burst of the GUV. The analysis of the EGCg-induced shape changes shows that binding of EGCg to the external monolayer of the GUV increases its membrane area, inducing an increase in its surface pressure. Small-angle X-ray scattering experiments indicate that the intermembrane distance of multilamellar vesicles of PC membrane greatly decreased at EGCg concentrations above the threshold. On the basis of these results, we discuss the mechanism of the EGCg-induced bursting of vesicles. [1] Biophys. J., 92, 3178-3194 (2007)

Abstracts

– Lipid biophysics –

P-149

Time-resolved X-ray diffraction studies of non-lamellar phase transitions in monoacylglycerols

T.-Y. D. Tang, O. Ces, R. H. Templer, J. M. Seddon
Department of Chemistry, Imperial College London, SW7 2AZ London, U.K.

The mechanisms of lamellar-cubic and cubic-cubic phase transitions in amphiphilic systems are poorly understood due to experimental problems with reproducibility and limited resolution of X-ray diffraction data. The relevance of non-lamellar phase transitions to dynamic processes in cell membranes is unclear, although it is generally accepted that they are intimately related to processes such as membrane fusion and fission. In order to address this issue in a well-defined model system, pressure-jump synchrotron X-ray diffraction was used to probe the kinetics of lyotropic phase transitions in pure lipid systems.
The problem of reproducibility has been overcome, and quasi-equilibrium and non-equilibrium studies have been undertaken on the lamellar to cubic transition of a novel amphiphilic system. The results obtained show similar trends to other monoacylglycerols, suggesting that such non-lamellar phase transitions may have a universal mechanism. Furthermore the effect of varying the pressure jump amplitude on the kinetics were investigated and shown to be comparable with that previously reported in other systems. Swollen cubic phases have been identified in equilibrium studies, suggesting that these phases are thermodynamically stable and may play a role in facilitating the phase transitions. The results illustrate the potential of X-ray pressure-jump technique for elucidation of phase transition pathways in defined model systems, and in the future may help to clarify the mechanisms underlying dynamic rearrangements in biomembranes.

P-150

Lipid diffusion in leaflets of supported bilayers: coupled or not coupled?

B. H. Tinland[1], C. Scomparin[2]
[1]CRMCN-CNRS, Marseille, France, [2]University of Aix-Marseille II, Marseille, France

Dynamics of Supported Lipid Bilayers (SLB) is of wide interest because it provides a convenient two-dimensional biomimetic system, located at a well-defined place which facilitates quantitative measurements. System properties can be tuned by playing with parameters such as roughness and chemical composition of the substrate, pH, ionic strength,.... It can be used as a model membrane, increasing the complexity of the system when adding cholesterol, proteins,... and also provides interesting perspective like designing bio-sensors.
As a starting point, it is crucial to have a good knowledge of the bilayer behavior itself. Here we want to address the question of the coupling or not coupling of the two leaflets. We present a study of the behavior of one component-symmetrical bilayers deposited by different means on glass or mica. Based on AFM observations and on diffusion coefficient measurements carried out during the gel-fluid transition, it shows in which conditions coupling of the two leaflets can occur. Therefore SLB can be described either as floating over the surface, leading to similar dynamic of both leaflets, close to the dynamics of free standing bilayer OR as presenting uncoupled leaflets: the proximal layer being more strongly adsorbed on the surface, lipids diffuse more slowly (x 10) than in the distal layer. Depending on the situation, this would affect the dynamics of any object that would be later inserted, like membrane proteins. Coupling or not coupling of the leaflets are explained regarding the roughness and/or the chemical composition of the substrate.

P-151

The Phase Behaviour of DPPC: 5-Androsten-3β-ol Systems

A. H. Trevenen, R. V. Law, J. M. Seddon, T. I. Magee
Department of Chemistry Imperial College London

Multinuclear MAS NMR spectroscopy (^{1}H, ^{2}H, ^{13}C and ^{31}P) and x-ray diffraction techniques have been employed to study the physical properties of androsten-3β-ol (Andro): dipalmitoylphosphatidylcholine (DPPC) mixtures in excess water. We have shown that Andro: DPPC forms a liquid ordered (L_o) phase at high Andro concentrations. Compositions used for these experiments range between 15-50 mol% Andro. Various properties were examined, including in-plane packing, deuterium order parameters of the lipid chains and motion of DPPC with Andro. These results were compared with that of Cholesterol and found that for the order parameters Andro: DPPC systems were less ordered for the whole methylene chain. Finally at low temperature evidence of a phase co-existence region, gel-type/L_o phase, similar to that seen in chol: DPPC mixtures, is seen in 50mol%Andro: DPPC.

P-152

Functional tBLMs as biosensor devices

I. K. Vockenroth, I. Köper
Max Planck Institute for Polymer Research, Mainz, Germany

Tethered bilayer lipid membranes (tBLM) have been shown to provide a powerful platform for the incorporation of membrane proteins in an artificial but quasi-natural environment. They are especially suited to study the incorporation and function of ion channels, where a high sealing resistance of the membrane is essential. The proximal leaflet of the bilayer is covalently coupled via a spacer group to a solid support, providing excellent mechanical stability (for weeks), combined with the conditions for protein incorporation such as fluidity and high electrical resistance. The coupling to a solid support allows the characterisation by surface analytical tools such as AFM, Surface Plasmon Resonance Spectroscopy and Electrochemical Impedance Spectroscopy.
The functional incorporation of the toxin α-Hemolysin was achieved. The tBLM provides a submembrane space between the solid support and the bilayer, yielding the ability to measure ion fluctuations through the incorporated channels and allowing the direct electronic read-out of changes happening in the cavity of the protein.
Downsizing the membrane area on μ-electrodes gave giga-ohmic resistances, showing sealing properties comparable to BLMs. This giga-seal allows for highly sensitive measurements of only a few channels with a patch clamp amplifier.
With this system a biosensor with modified biological receptors as actual sensing units is feasible, whereas the high stability opens the perspective of time-enhanced experiments and continuous monitoring. Thus a major step towards the use of proteins as stochastic sensing elements in possible biosensor applications has been accomplished.

Abstracts

– Lipid biophysics –

P-153

Interaction of porphyrin derivatives with unsaturated liposomes studied by EPR

I. Voszka[1], P. Gróf[1], G. Corradi[2], P. Maillard[3], H.-J. Steinhoff[4], G. Csík[1]

[1]Institute of Biophysics and Radiation Biology, Semmelweis University, Budapset, [2]Research Institute for Solid State Physics and Optics, Hungarian Academy of Sciences, Budapest, Hungary, [3]Institut Curie, Section de Biologie, Orsay, France, [4]Fachbereich Physik, Universität Osnabrück, Germany

Photodynamic treatment is a combination of visible light and light-absorbing chemicals. It is believed to be mediated by the generation of singlet oxygen. Due to the short half-life and short diffusion path of this species the primary damage is expected in the immediate vicinity of the photosensitizer in the membrane.

We examined the effect of molecular structure of porphyrin derivatives on their localization in the membrane structure. For our studies porphyrin derivatives with symmetrical and asymmetrical structure were selected. Liposomes containing unsaturated lipid were used to study the interaction. The localization of liposome-bound dyes was studied by electron paramagnetic resonance (EPR) spectroscopy. The efficiency of photodynamic treatment was followed by the decay of the EPR signal amplitude.

The porphyrin–lipid interaction strongly depends on the symmetry and polarity of porphyrin derivatives. For tetraphenyl porphyrins we propose that the asymmetrical derivative is situated much deeper within the membrane than the symmetrical one. Only unsaturated fatty acids were sensitive for the phototreatment, and porphyrins localized in closer connection with the double bond of the unsaturated fatty acid were more effective.

P-154

The regulation of phospholipase activity by lipid membrane structure

K. Wagner, G. Brezesinski, H. Möhwald

Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Langmuir monolayers of phospholipids are suitable models to study enzymatic reactions at interfaces because they allow us to easily manipulate many parameters in a defined way. The application of surface-sensitive techniques like grazing incidence X-ray diffraction and infrared reflection-absorption spectroscopy permits *in situ* observation of particular interactions that occur at biological membranes. The hydrolysis of phosphatidylcholines by different phospholipases was thus investigated.

I am presenting the latest results of the investigation of phospholipase D (PLD) and phospholipase A_2 (PLA_2) in dependence of the lipid monolayer structure. Briefly, PLD activity depends on the segregation of the hydrolysis product (phosphatidic acid, PA) within the monolayer. However, a specific structural parameter of the substrate-containing phase is not crucial for high activity. Instead, decisive structures of PA-rich domains cause an activation or inhibition of PLD.

PLA_2 exhibits maximum activity in the presence of liquid-expanded and condensed phase coexistence. Therefore, phase boundaries play a critical role in this process. Liquid-liquid immiscibility as found in mixed phospholipid/cholesterol monolayers is also sufficient to activate the enzyme. This finding is an important progress in the comparison of biophysical observations with the physiological conditions of biological membranes. There, liquid-ordered domains (rafts) eventually occur within liquid-disordered phases. The possible role of these domains in the regulation of membrane-associated proteins is discussed within the context of our study.

P-155

Rupturing and FRAP experiments of micro-BLMs on highly ordered porous silicon substrates

D. Weiskopf, C. Steinem

Institute of Organic and Biomolecular Chemistry, Tammannstr. 2, Georg-August University of Göttingen, 37077 Göttingen, Germany

We hypothesized that the continuous decrease in membrane resistance observed for nano-BLMs and micro-BLMs as monitored by impedance spectroscopy is a result of a continuous and independent rupturing of single membranes covering the pores of the porous material. To prove this hypotheses micro-BLMs were prepared on porous silicon substrates with a pore diameter of 5 μm. The upper surface of the porous material was coated with a gold layer followed by chemisorption of DPPTE. The lipid component used for the formation of micro-BLMs was DPhPC dissolved in *n*-decane. The rupturing process of micro-BLMs on the porous material was followed over time by means of fluorescence microscopy. Rupturing of bilayers covering the pores was detected by a loss in fluorescence, which was related to the continuous decrease in membrane resistance. Moreover, by means of FRAP, we investigated the dynamics of lipids in micro-BLMs to determine the lateral diffusion coefficient of the corresponding lipids. The principle of the FRAP-technique is to irreversibly photobleach a certain region inside a fluorescently labelled bilayer with a short intense light pulse. Immediately after bleaching, a weakened light beam from a Hg-lamp is used to measure the recovery of fluorescence within the bleached area as a result of diffusional exchange of bleached fluorophores by unbleached molecules from the proximity.

The porous silicon substrates were kindly provided by Dr. S. Dertinger (Infineon, Munich) and Dr. M. Bubolz (Bayer Technology Services GmbH, Leverkusen).

P-156

Clustering of Shiga Toxin - Lipid Reorganisation and Invaginations in Model Membranes

B. Windschiegl[1], W. Römer[2], L. Johannes[2], C. Steinem[1]

[1]Institut für Organische und Biomolekulare Chemie, Tammanstr. 2, Georg-August-Universität, 37077 Göttingen, Germany, [2]Laboratoire Trafic et Signalisation, Institut Curie, 26 rue d´Ulm, 75248 Paris Cedex 05, France

Shiga toxin (STx) is a member of the AB_5 class of bacterial protein toxins. The non-toxic B-subunit (STxB) binds specifically to the cell surface receptor globotriaosylceramide (Gb_3). Subsequently, the Gb_3-STx complex internalizes by endocytosis and can be transported via the retrograde route. To gather more information on this fundamental cellular process we focused on the first step of endocytosis – the binding of STxB to Gb_3 embedded in artificial membranes. Binding of STxB to giant unilamellar vesicles (GUVs) containing Gb_3 leads to the formation of invaginations. To better understand the bilayer deformation in GUVs we characterized in detail the STxB-Gb_3 interaction by means of solid supported lipid bilayers. We investigated the lateral organization of the protein on the bilayer and its influence on the organization of the lipids by scanning force and fluorescence microscopy. We were able to visualize a clustering of STxB and a protein-induced reorganization of the lipids in bilayers. The observed clustering of STxB, lipid phase separation and the invaginations in model membranes strongly depend on the lipid composition. In similar experiments with a monoclonal anti-Gb_3 IgM, clustering of the antibody on solid supported bilayers was also observed, however no invaginations occurred in GUVs. We hypothesize that specific STxB-Gb_3 interactions induce a lipid reorganization thus leading to the observed invagination process.

Abstracts

– Lipid biophysics –

P-157

Relationship between lipid peroxidation and phospholipases in biological membranes

M. V. Zamaraeva[1], E. T. Tokhtaeva[3], I. V. Dyachuk[2], Y. T. Timusheva[2], B. A. Salakhutdinov[2], E. A. Olchowik[1], O. N. Vagin[3]

[1]Dept. of Biophysics, University of Bialystok, Poland, [2]National University of Uzbekistan, Tashkent, Uzbekistan, [3]Dept. of Physiology UCLA, Los Angeles, USA

Induction of lipid peroxidation (LPO) in mitochondria and sarcoplasmic reticulum results in an increase in activity of phospholipases (PL) A, D and C. However, in the presence of LPO inducers, the rates of hydrolysis of endogenous oxidizible mitochondrial phospholipids or exogenous phospholipids unsusceptible to LPO were essentially identical. Thus, the increased activity of PL in the presence of LPO inducers can not be explained by the possibility that oxidized lipids are preferential substrates for PL. On the other hand, LPO is accompanied by the increase in rigidity of the hydrophilic regions of the membrane, and by the increase in fluidity of the hydrophobic ones as detected by ESR method, suggesting that changes in membrane structure induced by LPO may lead to stimulation of membrane PL. Also, treatment of mitochondrial membranes by exogenous PL A or activation of the endogenous PL both resulted in a suppression of LPO, which could be prevented by a PL inhibitor bromophenacyl bromide. Consistent with this observation, accumulation of LPO products in mitochondria saturated within 30 min after the addition of inducers of peroxidation. We conclude that activation of phospholipases in response to changes in membrane structure induced by LPO may form an auto-regulatory mechanism which can inhibit generation of free radicals.

P-158

Perturbation of bilayer structure by pHLIP

M. Zoonens, D. M. Engelman

Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, Connecticut, U.S.A.

The isolated C-helix of bacteriorhodopsin is water-soluble and unstructured above pH 7, and spontaneously inserts as a helix across lipid bilayers with a pK_{app} of 6[1]. The insertion of this peptide, dubbed pHLIP for pH Low Insertion Peptide, is pH-dependent, fully reversible, and oriented with the C-terminus across the membrane. pHLIP can be used as a tool for therapeutic drug delivery, inserting across the membrane and translocating a cargo molecule linked to the C-terminus via a disulfide bond. The reducing conditions of the cytoplasm then cause the release of the cargo molecule into the cell[2].

The thermodynamics of pHLIP binding and insertion in membranes was analysed in detail[1], however little is known about the kinetics of the insertion process. In solution, the soluble, bound and inserted forms of the peptide are in equilibrium. pH variations shift this equilibrium toward the membrane or toward the solution. In our work, the effects of the pHLIP binding on lipid bilayers were studied: we investigated membrane fluidity by fluorescence anisotropy and membrane permeability by following the passive diffusion of an aromatic acid, such as salicylic acid (SA) through a POPC bilayer in a liposomal assay[3]: Tb^{3+} loaded vesicles were incubated with SA and under excitation at 318 nm, the enhanced emission of Tb^{3+} luminescence at 545 nm reveals the formation of SA/Tb^{3+} complexes.

1. Hunt *et al*., (1997) *Biochemistry*, 36, 15177-92
2.Reshetnyak *et al*., (2006) *PNAS*, 103, 6460-65
3.Thomae *et al.*, (2005) *Biophysical J.*, 89, 1802-11

Abstracts

– Membrane protein folding –

P-159

ATR-FTIR spectroscopy of membrane bound Ras protein

Y. Adigüzel[1], C. Kötting[1], J. Kuhlmann[2], H. Waldmann[2], K. Gerwert[1]
[1]Biophysics Department, Ruhr university, Bochum, Germany, [2]Max Planck Institute of Molecular Physiology, Dortmund, Germany

We looked at the structural and functional properties of the membrane bound Ras protein on solid supported model membranes by means of ATR-FTIR spectroscopy. FTIR is a non-invasive technique and reveals the vibrational energies' related information (i.e. bond orders, electrostatic interactions, H-bonding, charge distributions, protonation states and dynamics) in a sample. ATR-FTIR is a surface sensitive application of this technique utilizing the entrapment of light entering a medium with higher refractive index, below a critical angle. Via ATR-FTIR, eventual interaction of the sample with the evanescent wave arousing from the points of reflection towards the sample is investigated. Ras protein takes role in the signal transduction of cellular events such as cell division, proliferation and apoptosis. During signal transmission, it is bound to the inner leaflet of the cellular membrane and shows activity through a GTP-bound to GDP-bound switch mechanism. Its malfunctioning may lead to cancer. Most of the former studies carried out on the truncated form of the protein which is excluding the membrane binding region with the lipid anchors. Ras lipid anchoring peptide region or the full length protein with the lipid anchors bound to the membrane were recently started to be investigated. Revealing the structural, functional features and dynamic interactions with its effectors and regulators of the membrane bound Ras protein may pave new grounds for therapeutically significant solutions to cancer.

P-160

Biophysical factors in drug action

E. Albert
University of Medicine and Pharmacy, Tîrgu Mures, Romania

One of chief difficulties in seeking a rational explanation of the biological activities of drugs in terms of simple physicochemical or biophysical factors is the apparent simplicity of the relationships which may readily be deduced by analogy with artificial model systems. The justification for the use of such models has frequently been based on the assumption that the living system is so complex that the gross properties of a particular structure are often embodied in a simplified reconstructed system.. For the organization of living matter frequently takes the form of discrete cellular fabrics or membranes, and, apart from the permeability of such membranes, the uptake of a drug is also influenced by the asymmetrical forces resident at their surfaces of separation. Lipids and proteins can be spread on suitable substrates as two-dimensional films, or monolayers. The changes in the physical state, surface pressure and surface potential of the monolayers, gives an accurate measure of the associating forces between the biological components and the drugs which are introduced into the underlaying substrates. In the analytical approach to the nature of drud action, the bulk of existing pharmacological data can be interpreted by assuming that drugs combine with hypothetical receptors in the living organisms to produce similar or antagonistic responses. When this occurs it is supposed that the drugs compete for the same receptors in the surface or tissue which is the site of drug action.

P-161

Architecture and effects on lipid bilayer of two staphylococcal oligomeric pores by AFM

A. Alessandrini[1], G. Prevost[2], M. Dalla Serra[3], G. Viero[3]
[1]CNR-INFM-S3 Nat. Res. Center on nanoStructures and bioSystems at Surfaces, Modena, Italy, [2]Institut de Bactériologie, UPRES EA-3432, Strasbourg, France, [3]CNR-ITC Istituto di Biofisica Sezione di Trento and University of Trento, Dept. of Physics, Italy

Staphylococcus aureus produces several β-barrel pore forming toxins (PFT). Among these γ-hemolysins and α-toxin are secreted as water-soluble monomers that assemble into oligomeric pores on membranes. The bicomponent hemolysins form a pore by the association of two proteins and are related in term of 3-D structure to the well characterised α–toxin. There are not any available 3D informations on the hemolysin pore. Here, we used Atomic Force Microscopy to compare the architecture of the hemolysin hetero-oligomer and the α-toxin homo-heptameric pore. AFM images revealed the presence of ring shaped hemolysin oligomers on the surface of lipid bilayers and it was possible to obtain structural parameters of the hemolysin oligomer. These results were compared to the crystallographic and experimental data obtained for α-toxin. Despite a similar structural organization of α-toxin and hemolysin, a different stoichiometry is suggested. Surprisingly, the toxins caused different effects on the bilayer. Hemolysin strongly disorganized the bilayer with formation of oligomers clusters. On the contrary, neither the disruption of the lipids nor the clustering was observed with α-toxin. The different behaviour of these toxins on the lipid bilayer organization has been confirmed by Dynamic Light Scattering. These results may suggest that microheterogenities in structural arrangements could be responsible for different effect of PFT on membranes and perhaps for different cell specifity.

O-162

Unfolding and refolding of a predominantly α-helical, tetrameric potassium channel protein

F. N. Barrera, L. Renart, M. L. Molina, J. A. Poveda, J. A. Encinar, A. M. Fernández, J. L. Neira, J. M. González-Ros
Instituto de Biología Molecular y Celular. Universidad Miguel Hernández. Elche, 03202 Alicante, Spain

The prokaryotic potassium channel KcsA has been used as a model to study the folding "in vitro" of an oligomeric, alpha-helical membrane protein. A study in plain detergent solution (Barrera et al. Biochemistry (2005) 44, 14344) established that increasing concentrations of 2,2,2-trifluoroethanol (TFE) causes two successive, protein-concentration dependent, cooperative transitions. At moderate TFE concentrations, the tetrameric protein partly looses its secondary structure and dissociates into monomers. Such effects on the protein structure can be significantly reversed by dilution into TFE-free media. On the contrary, further increasing the TFE concentration caused that the partly unfolded monomer underwent an irreversible denaturation.
The effects of TFE on the protein structure have been monitored in mixed detergent-lipid micelles instead of plain detergent solutions. We find that the presence of specific lipids causes that the tetramer to monomer transition from above, which occurs at similar TFE concentrations, becomes now fully reversible. This observation lends support to a new notion that specific lipids play a role in the folding and oligomerization "in vitro" of membrane proteins. Also, we detected an additional, reversible cooperative transition at lower TFE concentration, which involves dissociation of clusters (supramolecular assemblies of several KcsA tetramers) into individual tetramers. Such clusters arefunctionally significant both "in vitro" and "in vivo" and their assembly seems to cause little or no changes in the secondary structure of the protein.

Abstracts

– Membrane protein folding –

P-163

Protein self-assembly and lipid binding in the folding of the potassium channel KcsA

F. N. Barrera, M. L. Renart, M. L. Molina, J. A. Poveda, J. A. Encinar, J. L. Neira, J. M. González-Ros
Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche, 03202 Alicante, Spain.

The potassium channel KcsA is a bacterial tetrameric protein which function is modulated by its self-association in clusters. KcsA solubilized in the detergent dodecyl maltoside (DDM) monomerizes and unfolds in the presence of moderate concentrations of the alcohol 2,2,2-trifluoroethanol (TFE), in a partially irreversible process (Barrera *et al*. (2005) Biochemistry 44 (43), 14344-52). We have observed that the transition from the folded tetramer to the unfolded monomer becomes completely reversible when KcsA is solubilized in mixed micelles composed of the detergent DDM and the lipids DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) and DOPG (1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]). This finding allowed the determination of the standard free energy of the KcsA tetramer denaturation (30 kcal·mol^{-1}). Our results suggest that the lipids act as cofactors in the tetramerization of KcsA. We also observed that prior to the unfolding of the tetramer, the presence of low TFE concentrations caused the disassembly of clusters of KcsA channels, and that the level of clustering was not influenced by the presence of solubilized lipids.

P-164

Conformational transitions of human α-synuclein associated with phospholipid membranes

C. R. Bodner[1], S.-T.-D. Hsu[2], J. Christodoulou[2], C. M. Dobson[2], A. Bax[1]
[1]Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD, USA, [2]Department of Chemistry, University of Cambridge, Cambridge, UK

Fibrillar aggregates of the pre-synaptic protein alpha-synuclein (αS) are the primary components of Lewy bodies, the characteristic lesions of Parkinson's disease. Conformational transitions of intrinsically disordered αS in the presence of lipid membranes are likely to be crucial to the protein's native and pathological roles. Here we investigate the structure and biphysics of αS in the presence of isotropic lipid bicelles. Lipid bicelles offer the advantage of a natural lipid bilayer while also featuring small vesicle radii which render them amenable to solution NMR studies. In agreement with previous studies, our data shows that αS adopts helical structure in the presence of acidic phospholipids, presumably due to electrostatic interactions. However, we present evidence for further specific interactions between αS and bilayers containing phosphatidylserine (PS). Solution NMR shows that αS participates in dynamic exchange processes in association with the PS lipid bilayer, and complementary fluorescence and imaging data demonstrate that this interaction is coupled with enhanced aggregation propensity of αS. Finally, we show that in addition to the structural changes induced in αS, the bilayer is also altered by the association. ^{31}P NMR reveals accelerated hydrolysis of PS and perturbations to the geometry of the lipid bicelle when incubated with αS.

O-165

Manipulating the assembly of transmembrane helical proteins

P. J. Booth
Department of Biochemistry, University of Bristol, U.K.

Efficient systems need to be developed to unfold and re-fold alpha helical membrane proteins. We have been developing in vitro lipid-bilayer folding systems for membrane proteins. The stored curvature elastic stress of model bilayers can be used to optimise the rate and yield of folded protein. We have shown that events such as transmembrane helix insertion, as well as tertiary and quaternary structure formation are altered by the stored curvature stress of the bilayer. The curvature stress of a phosphatoidylcholine lipid bilayer can be altered by changing either the lipid chains or introducing a different headgroup such as phosphoethanolamine. We also find a more specific enhancement of folding by phosphatidylglycerol lipids.

P-166

Negatively charged lipid membranes induce fibril formation of Medin via an α-helical intermediate.

T. Borowik[1], G. Gröbner[2], A. Olofsson[3]
[1]Institute of Physics, Wroclaw University of Technology; Wroclaw, Poland, [2]Department of Biophysical Chemistry, Umeå University, Umeå, Sweden, [3]Department of Molecular Biology, Umeå University, Umeå, Sweden

Medin, 50-amino acid long internal fragment of lactadherin, was recently found to be the main constituent of aortic medial amyloid. This most common human amyloid occurs in over 95% of human population over sixty and is believed to contribute to age-related diminished elasticity of blood vessels. In this study, for the first time, the role of lipids in the aggregation of medin peptide was investigated.
In solution the peptide adopts random-coli structure with high fraction of β-sheets. Addition of lipid vesicles containing negatively charged phosphatidylglycerol (PG) shifts the β-sheet fraction into an α-helical one. Incubation of the peptide in solution or with neutral phosphatidylcholine (PC) vesicles does not induce its secondary structure change. However, the presence of negative surface charge on lipid vesicles shifts the structural equilibrium towards the β-sheet-rich conformation. This can be directly interpreted as the peptide aggregation into the fibril-like structures, as shown by atomic force microscopy. The driving force of medin membrane adsorption and its accelerated aggregation was found to be an electrostatic interaction between medin positively charged amino acids and negatively charged PG. The partition enthalpy of the peptide into the negatively charged lipid vesicles was determined by means of isothermal titration calorimetry to be twice as much as into the neutral ones.

Abstracts

– Membrane protein folding –

P-167

Molecular interactions versus local conformational details: TFE effects on bovine serum albumin.

R. Carrotta[1], M. Manno[2], P. L. San Biagio[2]

[1]DSFA, Via Archirafi 36, Palermo, 90123, Italy,, [2]IBF CNR, Via Ugo La Malfa 153, Palermo, 90146, Italy

The intertwining between intermolecular protein interactions solvent mediated and protein conformational details is of fundamental importance in the understanding of protein aggregation processes. Tri-Fluor-Ethanol (TFE) in solution can alter protein conformation, by interfering with hydrogen bonds and by enhancing the stability of the alpha helical secondary structure. In order to investigate the relation between intermolecular interaction and molecular conformation, we study the influence of TFE on the thermodynamic stability and on the conformational changes of a model protein, bovine serum albumin (BSA). Solvent mediated pair-wise interactions are investigated by static and dynamic light scattering, which give a measure of the second virial coefficient (*b2*) and of the hydrodynamic coefficient (*h*), respectively. F-UV and N-UV circular dichroism, steady-state fluorescence measurements from an internal (Triptophan) and an external probe (8-anilino-1-naphthalenesulphonate, ANS), inform on the protein conformational changes at different TFE concentrations. Results show that moderate concentration of TFE (less than 10%) can alter the molecular conformation of BSA. At TFE concentrations higher than 15%, BSA conformation is further changed and an effective attractive interaction is turned on, as indicated by light scattering data.

P-168

Polymer-mediated refolding of bacteriorhodopsin

T. Dahmane[1], F. Wien[2], Y. Gohon[1], J.-L. Popot[1]

[1]IBPC, UMR 7099 CNRS/Université Paris 7, [2]Soleil Synchrotron, L´Orme des Merisiers, Saint-Aubin, France

Among the major obstacles to pharmacological and structural studies of integral membrane proteins (MPs) are their natural scarcity and the difficulty to overproduce them under their native form. MPs can be overexpressed in non-native state as inclusion bodies, but inducing them to achieve their functional three-dimensional structure has proven a major challenge. We have recently established that an amphipathic polymer, amphipol A8-35, provides a favorable environment to fold to their native state three MPs, including bacteriorhodopsin (BR), the light-driven proton pump from the plasma membrane of Halobacterium salinarium (1). Amphipols, which are extremely mild surfactants, appear to favor the formation of native intramolecular protein/protein interactions over intermolecular or protein/surfactant ones. **Synchrotron radiation circular dichroism** (SRCD) presents over conventional CD a number of advantages, including a greater sensitivity, an improved signal-to-noise ratio, the possibility to work on more concentrated, very small samples (μl) and attainable shorter wavelength ranges at higher fluxes. It has been used here to compare the secondary structure of i) native BR trapped with A8 35, ii) BR refolded in A8 35 in the presence or iii) the absence of lipids, and iv) refolded, delipidated bacterio-opsin (dBO). The four spectra are found to be strictly identical down to ∼180 nm, indicating that refolded dBO has regained the secondary structure of the native holoprotein. SRCD has also been used to examine events that precede renaturation and the thermal stability of the various forms of native and refolded protein.

1. Pocanschi et al. (2006) Biochemistry 45, 13954-13961.

P-169

Characterisation of protein biopharmaceutical products and other biomacromolecules

A. Damianoglou[1], M. R. Hicks[1], T. R. Dafforn[2], A. Rodger[1]

[1]University of Warwick, U.K., [2]University of Birmingham, U.K.

The macromolecular biopharmaceutical active ingredients in available drugs range from simple single protein molecules such as monoclonal antibodies, to complexes formulated in vehicles designed for targeted delivery. In this project we focus on developing linear dichroism (LD, the difference in absorption of light polarised parallel and perpendicular to an orientation axis) and circular dichroism (CD, the difference in absorption of left and right circularly polarised light) spectroscopy into methods for characterising biopharmaceuticals with the ultimate aim of them being used for batch characterisation and/or diagnostic tools in clinical biochemistry.

The first goal has been to establish stable multiple wavelength calibration standards for CD spectropolarimeters. We now have chemically and enantiomerically pure stable standards which are available in both mirror image forms. CD and infra red (IR) spectroscopy have been used to facilitate characterisation of the secondary structure of soluble proteins.

Membrane proteins are more challenging samples to work with than water soluble globular proteins. We are developing LD to complement CD and IR for these systems. General conclusions are obtained from comparing the spectra of membrane proteins prepared by different methods. CD, LD, IR, Light Scattering and Fluorescence spectroscopy as well as Size Exclusion Chromatography have been used to characterise the structure of membrane-bound proteins and their insertion into model membrane systems.

P-170

Protein interactions in the membrane: Exploring the role of transmembrane helices in disease

A. M. Dixon

University of Warwick, Coventry, U.K.

A key element of a cell is its lipid membrane; as well as sequestering the contents of the cell, proteins embedded in the membrane regulate a large number of biological processes via a carefully orchestrated series of protein-protein interactions. Such interactions are well characterised in the case of soluble domains, but little detail is known about protein interactions in the plane of the membrane. Thus far, intensive studies of proteins such as Bacteriorhodopsin and Glycophorin A have lead to the development of simple models for α-helical membrane protein folding and interactions; one well-known example being the two-stage model, which describes the lateral association of helical transmembrane (TM) domains.

We have used these models as a foundation upon which to build an understanding of TM helix interactions implicated in key cellular functions and the development of disease. Using a wide variety of biochemical and biophysical techniques (AUC, MS, NMR) to study membrane protein complexes involved in immune response and cancer, we have observed strong and specific interactions between TM domains via networks of non-covalent interactions (e.g. hydrogen bonding, electrostatic interactions). In these systems we see the behaviour of the TM domains, with respect to oligomerisation and complex formation, closely mirroring that of the full length proteins suggesting that the TM domains play a significant energetic role in driving the formation of membrane protein complexes. These biologically important examples provide new illustrations of key concepts in membrane protein folding, and in some cases have lead to revision of accepted mechanisms of interaction and function.

Abstracts

– Membrane protein folding –

P-171

Structural dynamics in recombinant light-harvesting chlorophyll a/b complex as monitored by EPR

C. Dockter[1], A. Volkov[2], Y. Polyhach[2], G. Jeschke[2], H. Paulsen[1]
[1]Institut für Allgemeine Botanik, Johannes-Gutenberg-Universität Mainz, Germany, [2]Max Planck Institut für Polymerforschung Mainz, Germany

Light-harvesting chlorophyll *a/b* complex (LHCIIb) is a major component of the photosynthetic apparatus in green plants. Its 232-amino acid apoprotein noncovalently binds 8 chlorophyll (Chl) *a* and 6 Chl *b* molecules as well as four carotinoids. An astonishing feature of this protein is that it spontaneously folds into its three-dimensional structure *in vitro* upon mixing the denatured protein with its cofactors in detergent solution.
In order to study the structural dynamics of LHCIIb, we combine site-directed spin labeling with electron paramagnetic resonance spectroscopy (EPR), specifically CW, ESE and DEER (double electron electron resonance), a pulse EPR technique that allows to measure distances in the range from 2 to 6 nm. By using these techniques we were able to assess local mobilities and accessibilities to hydrophilic or hydrophobic quenchers of individual protein domains in LHCIIb and changes in the distance distribution between two spin labels attached to selected amino acid residues.
Moreover, in this work we combine pulse EPR methods for the first time with freeze-quench techniques to gather structural and kinetic information during the folding of LHCIIb in the time range from ms to min. The results will contribute to a better understanding of the dynamics and the folding mechanism of membrane proteins and of the self-organisation of supramolecular assemblies.

P-172

EPR studies on a HAMP domain of the archaeal phototransducer NpHtrII

M. Doebber[1], E. Bordignon[1], J. P. Klare[1], M. Engelhard[2], H.-J. Steinhoff[1]
[1]Universitaet Osnabrueck, Osnabrueck, Germany, [2]MPI fuer Molekulare Physiologie, Dortmund, Germany

The photosensitive unit triggering the negative phototaxis in *Natronomonas pharaonis* is formed by the receptor sensory rhodopsin II (NpSRII) and its cognate transducer (NpHtrII) in a 2:2 stoichiometry. Upon light excitation, a displacement of helix F in the receptor is thought to trigger a rotation of TM2 in the transducer. This signal is then transmitted to the cytoplasmic part of the transducer in a still unknown mechanism.
Structural information exists for the transmembrane region of this complex as well as for the rod shaped cytoplasmic part of NpHtrII. The linker between the membrane anchor and the cytoplasmic signaling domain contains two so called HAMP domains which are structurally and functionally not characterized. Therefore, an EPR investigation with site-directed spin labeled transducers in the NpSRII-HtrII complex has been carried out.
Thirty positions in the first HAMP domain of the transducer have been spin labeled and investigated via cw EPR spectroscopy in order to get information about the structure and the topology of this domain. Studies were performed for the complex in the detergent-solubilized and in the lipid-reconstituted state. Low temperature EPR measurements gave insights into the transducer-transducer and receptor-transducer interactions in the 2:2 complex, respectively. Additionally, changes in the salt concentration as well as in the temperature monitored the effects on the two spectral components present in the RT EPR spectra.

P-173

Correlation between the aggregation behaviour of Bcl-xL and its membrane-active properties

G. Fuertes, C. Schlemmer, J. Salgado
Molecular Science Institute, University of Valencia, Paterna, Spain

The anti-apoptotic protein Bcl-xL, a member of the Bcl-2 family, performs its function at the outer mitochondrial membrane where it inhibits pro-apoptotic counterparts through unknown mechanisms. Pro-apoptotic proteins of the same family, like Bax, possess pore-forming capability permitting the release of apoptotic factors from mitochondria. A pair of central amphipathic α-helices, α5α6, appears to be the minimum motif needed to permeabilize membranes. This domain is also present in Bcl-xL with a high level of homology with that of Bax-type proteins. We have studied both full-length Bcl-xL and fragments derived from the central helices. We show that Bcl-xL has also membrane-destabilizing effects and is able to release small probes encapsulated into model membrane systems but only at acidic pH. In parallel, we have estimated the size of the proteins at different pHs. According to light scattering measurements, Bcl-xL is dimeric at neutral pH, but associates into large aggregates at acidic pH. In contrast, Bcl-xL α5α6 is already aggregated at pH 7 and oligomerizes further as the pH is lowered. These proteins seem to partition into liposomes only at acidic pH and the size of vesicles upon addition of proteins remains unaffected. Taken together these results suggest that Bcl-xL is able to form pores of moderate size in lipid bilayers at acidic pH and the acquisition of an oligomeric structure may be involved in the pore-forming activity. On the other hand, at neutral pH, Bcl-xL does not cause leakage from vesicles, probably because a positive net charge in the protein is required for an appropriate interaction with the membrane lipids.

P-174

Kinetics of membrane-peptide insertion

M. R. Hicks[1], T. R. Dafforn[2], A. Damianoglou[1], A. Rodger[1]
[1]University of Warwick, UK, [2]University of Birmingham, UK

The interaction of peptides and proteins with membranes is an important biological and pharmacological process. Measuring the kinetics of spontaneous insertion of peptides into membranes can be carried out using different techniques, for example fluorescence and circular dichroism (CD) spectroscopy are commonly used. If the events are fast, then combining these techniques with stopped flow enables one to follow these events down to millisecond timescales. However, these techniques rely on the changing signal due to either a change in environment (usually polar to apolar) or a change in the fold of the peptide. The important information on the orientation of the peptide in the membrane is not accessible.
Here we present data on the insertion of the peptide antibiotic gramicidin into membranes, monitored using a combination of biophysical techniques including the novel approach of fast, micro-volume linear dichroism spectroscopy (LD). LD is the difference in absorbance of light polarised parallel and perpendicular to an orientation axis. There are many benefits to using LD. One of the main advantages is that one can gain information on the orientation of the peptides and proteins within the membrane. In addition, the kinetics of the process can be followed in real time with changes in orientation of different chromophores giving information that can be used to elucidate the mechanisms of action. A model for gramicidin insertion into the membrane is suggested and the general applicability of this technique is discussed.

Abstracts

– Membrane protein folding –

P-175

Membrane Reconstitution of Human Neuropeptide Y Receptor Type 2

I. Hirsch, P. Schmidt, C. Montag, R. Rudolph, D. Huster
Institute of Biotechnology, MLU Halle-Wittenberg

The human neuropeptide Y receptor type 2 is a member of the G protein-coupled receptor family (GPCRs), also known as seven transmembrane α-helix receptors. It is abundant in the hippocampus and has a dampening effect on brain activity. This highly conserved receptor has a binding site for neuropeptide Y (NPY), a 36 amino acid peptide neurotransmitter found in the brain and autonomic nervous system. Investigation of receptor structure in modell membranes by Solide-State NMR is of great importance for development of new drugs.
The Y2 receptor was overexpressed in *E. coli* as inclusion bodies using high-density-fermentation. After cell lysis the material was harvested by solid-liquid separation, solubilized with denaturating detergents, purified using immobilized metal ion affinity chromatography. The purified receptor was refolded *in vitro* in the presents of non-denaturating detergents. Fluorescence measurements were performed to show specific binding of NPY to the the receptor in this micelle state.
For the reconstitution of the receptor in liposomes we used the dilution method. Therefore the Y2 receptor was incubated with n-Oktyl ß-D-glycopyranosid pre-treated liposomes containing POPC, POPS, and cholesterol. All detergents were removed by dialysis.
The first results of receptor function we performed point towards an active conformation of the Y2 receptor in the liposomes. Using fluorescence titration experiment we were able to show affinity of the ligand to the receptor in the low nanomolar range.

P-176

Antimicrobial Cateslytin promotes rigid domains when interacting as beta-sheet on negative membranes

F. Jean-Francois[1], P. Bergson[2], S. Castano[1], M.-H. Metz-Boutigue[3], P. Vacher[2], J. Elezgaray[1], M. Roux[4], E. J. Dufourc[1]
[1]UMR 5248 CBMN-Université bordeaux 1-ENITAB, IECB, 33607 Pessac, France, [2]INSERM 347, Bordeaux, France, [3]INSERM 575, Strasbourg, France, [4]CEA-Saclay, Gif-sur-Yvette, France

The antimicrobial peptide Cateslytin (bCGA RSMRLSFRARGYGFR) is a five-positively charged arginin rich peptide known to inhibit the release of catecholamine in chromaffin granules. Although it is able to rapidly cross the bacterial membrane and stop the bacterial growth, the mechanism of action has not been studied yet. In order to better understand both targeting and selectivity towards microorganisms, model membranes with neutral or negative global net charge have been chosen to respectively mimic bacterial or mammalian membranes. Structural studies have been performed using polarised ATR-FTIR, circular dichroïsm and high resolution NMR. Membrane dynamics has been followed using deuterium labelled lipids and solid state NMR. Patch clamp experiments were also performed on lipid vesicles to measure channel conductivity. All-atom molecular dynamics on hydrated peptide-lipid membrane systems was also used to assess the interaction from the atomic level. Main results from this interdisciplinary approach are threefold. i) Electric current passages through membranes demonstrate permeation akin to pore formation. ii) Peptide-induced formation of rigid domains mainly made of negatively charged lipids is found. iii) Peptide antiparallel β-sheets are observed preferentially with negatively charged lipids. The general picture leads to the proposal that membrane destabilization/permeation is promoted by rigid domains stabilised by peptide β-sheets.

P-177

Structural Studies of Carnitine Palmitoyltransferase I

Z. Jenei, A. M. Dixon
University of Warwick, Department of Chemistry, Coventry, CV4 7AL, U.K.

The members of the carnitine palmitoyltransferase I (CPT1) family are polytopic integral membrane proteins in the outer membrane of mitochondria. CPT1 is the key regulation enzyme for the fatty acid import system. The two catalytically active isoforms, CPT1A (found in liver) and CPT1B (found in muscle), catalyze the initial step of fatty acid import by facilitating the transport of the long and medium chain acyl-CoA into the mitochondrial matrix. Enzyme inhibition by malonyl-CoA is a primary control point for this process.
CPT1A and CPT1B share a high degree of sequence similarity (65%), however they differ significantly in their inhibitor binding kinetics and their ability to adapt kinetic properties to various physiological states. It has been suggested that these differences in inhibitor binding are due to differences in enzyme structure, specifically in interactions between N- and C-terminal domains. Furthermore, in one isoform, the transmembrane (TM) domains are also thought to play a key role in modulating these interactions, suggesting that TM domain interactions differ in the two isoforms.
We have investigated in more detail the interactions between individual domains of liver- and muscle-type CPT1 enzymes in order to better understand the structural features that underlie differences in inhibitor binding. The structure and interactions of soluble and membrane-spanning domains have been investigated using a variety of biochemical and biophysical techniques to determine oligomeric state, secondary structure, and binding.

P-178

A role for transmembrane domains in the formation of MHC Class II /Invariant Chain complexes

G. W. King, A. M. Dixon
University of Warwick, Department of Chemistry, Coventry, U.K.

A crucial step in antigen presentation is the transport of Major Histocompatibility Complex Class II (MHC) molecules via interactions with Class II-Associated Invariant Chain (Ii). Ii is a 216 residue membrane protein that forms a homotrimer and subsequently binds three MHC Class II α/β heterodimers (composed of two glycosylated membrane proteins, α and β, that are 230 and 240 residues long respectively) to form a nonomeric complex.
Recent studies implicate the transmembrane domain (TM) of Ii in correct assembly and function of the MHC/Ii complex. Mutagenesis of the Ii TM has been shown to disrupt complex formation and antigen presentation. The Ii TM in isolation is a strongly associating trimer, and mutagenesis of the TM has revealed the importance of polar residues in stabilising the trimer, thus linking disruption of TM helix interactions to previously reported losses of Ii function. The TM domains of MHC Class II proteins are also thought to play a role; they have been shown to be important for intracellular trafficking and antigen presentation. Collectively, these results have lead to the proposal of a revised model for MHC/Ii complex formation which includes contributions from the TM domain.
In order to refine this model we have investigated the interactions between the TMs of Ii, MHCα, and MHCβ. These interactions have been studied using a range of biochemical and biophysical methods to probe oligomeric state, secondary structure, and the energetics of association *in vitro* and *in vivo*. Preliminary results lend further support to our model of MHC/Ii complex formation and also suggest specific sequence motifs that can perturb this association.

Abstracts

– Membrane protein folding –

P-179

Molecular mechanism of facilitated transport across biomembranes

J.-H. Lin[1], K.-L. Kuo[2]
[1]Divion of Mechanics, Research Center for Applied Sciences & Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]School of Pharmacy, National Taiwan University, Taipei, Taiwan

Passive transport of glucose transport across the plasma membrane is facilitated by members of the glucose transporter (GLUT/SLC2A) family. GLUT1, also known as the red blood cell glucose transporter, is one of the most extensively studied membrane transporters. However, the molecular mechanism of GLUT1-mediated transport is still largely unknown, partly due to the difficulty of obtaining a high resolution structure of this membrane protein. Here I will demonstrate how the homology model of GLUT1 can be refined by molecular dynamics simulations in the full-hydrated POPC bilayer using an iterative umbrella sampling scheme. The refined structure is in much better agreement with the chemical cross-linking experiments. Docking of glucose and a competitive inhibitor also predict the binding site which is in excellent agreement with previous mutagenesis experiments. The glucose transport pathways were then predicted by a novel docking scheme, named, SLITHER, which can locate a series of juxtaposed ligand binding sites with such membrane channels, along with the most favorable conformation at each binding site. Detailed knowledge of glucose transport will lead to advances in the understanding and designing the therapeutics of glucose homeostasis disorders, including type 2 diabetes mellitus.

P-180

Influence of the lipid composition on the cleavage of liposome-reconstituted APP(684-726) by trypsin

M. Marenchino, S. Murri, P. T. F. Williamson, G. Zandomeneghi, B. H. Meier, H. Wunderli-Allenspach, S. D. Krämer
ETH Zürich, Zürich, Switzerland

The cleavage of the transmembrane amyloid precursor protein (APP) by α-secretase prevents the processing of the protein to amyloid β, a hallmark in Alzheimer's disease. We were interested whether the membrane composition has an influence on the accessibility of the α-cleavage site of APP. The 5-carboxyfluorescein-labeled sequence APP(684-726) including the α-cleavage site, the transmembrane domain and 3 cytosolic histidines was reconstituted into liposomes of dimyristoyl phosphatidylcholine (DMPC), distearoyl PC (DMPC), egg PC and sphingomyelin (Sph), respectively. The liposomes were incubated at peptide concentrations between 0.5 and 80 μM with 0.5 nM trypsin which cleaves at the α-cleavage site of the peptide. Samples were collected at various time points and the fluorescent cleavage product was quantified by HPLC. The cleavage rates (v) were plotted against the peptide concentration ([S]). APP in 1% acetonitrile, in DSPC and in Sph showed typical Michaelis Menten behavior in the investigated concentration range. DMPC and egg PC revealed a linear relationship between v and [S]. The (initial) slope of v *vs* [S] (V_{max}/K_M in the systems following Michaelis Menten kinetics) was similar in all systems, though V_{max} and K_M varied significantly. This behavior is typical for changes in the catalytic process (*e.g.*, uncompetitive inhibition). According to this model, the systems with longer lipid acyl chains (DSPC, Sph) have slower catalytic rate constants while the systems with shorter acyl chains (DMPC, eggPC) seem to have higher catalytic rate constants.

P-181

Omiganan interaction with bacterial membrane models. Saturation as a potential trigger for activity.

M. N. Melo, M. M. Castanho
Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

The interaction of the dodecapeptide antimicrobial peptide Omiganan pentahydrochloride (ILRWPWWPWRRK-NH_2·5Cl) with bacterial and mammalian model membranes was characterized by means of UV-Vis absorption and fluorescence emission spectroscopy using large unilamellar vesicles of different proportions of POPC and POPG as models. Very high molar ratio partition constants ($(18.9\pm1.3)x10^3$ and $(43.5\pm8.7)x10^3$) were obtained for the bacterial models (POPG:POPC 4:1 and 2:1, respectively), these being about one order of magnitude greater than the partition constants obtained for the less anionic mammalian model systems ($(3.7\pm0.4)x10^3$ for the 100% POPC system). At low lipid:peptide ratios there were significant deviations from the usual hyperbolic-like partition behavior of peptide vesicle titration curves, especially in the case of the most anionic systems. Membrane saturation was shown to be related to such observations and mathematical models were derived to further characterize the peptide-lipid interaction under these conditions. The calculated peptide-to-lipid saturation proportions, together with the determined partition constants, suggest that the Minimal Inhibitory Concentrations of Omiganan pentahydrochloride could represent the conditions required for bacterial membrane saturation to occur.

O-182

Refolding SDS-denatured proteins by the addition of amphipathic cosolvents

C. Michaux[1], N. Pomroy[2], L. Chen[2], G. G. Prive[3]
[1]CPTS, Laboratoire de Chimie Biologique Structurale, FUNDP, Namur, Belgium, [2]Division of Cancer Genomics and Proteomics, Ontario Cancer Institute, Toronto, Ontario, Canada, [3]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

The refolding of proteins from the denatured state is of considerable practical and theoretical interest. A common situation is the refolding of proteins from urea or guanidinium solutions, but the process often fails due to the high propensity of proteins to aggregate upon the removal of the denaturant. Sodium dodecyl sulfate (SDS) is a highly effective and widely used protein denaturant. We show that certain amphipathic cosolvents such as 2-methyl-2,4-pentanediol (MPD) can protect proteins from SDS denaturation, and in several cases can refold proteins from the SDS-denatured state. This cosolvent effect is observed with integral membrane proteins and soluble proteins from either the α-helical or the β-sheet structural classes. MPD and related amphipathic cosolvents can modulate the denaturing properties of SDS, and we describe here a simple and effective method to recover refolded, active protein from the SDS-denatured state.

Abstracts

– Membrane protein folding –

P-183

Recombinant production and in vitro folding of cholecystokinin type A receptor

C. Montag, P. Schmidt, C. Berger, R. Rudolph, D. Huster
Institute of Biotechnology, MLU Halle-Wittenberg, Germany

The human cholecystokinin type A receptor is a seven transmembrane helical protein involved in cellular signal transduction. It comprisis one disulfide bond and belongs to the class A G protein-coupled receptors (GPCRs). The receptor mediates pancreatic growth and enzyme secretion, smooth muscle contraction of the gall bladder and stomach. GPCRs are targets for more than 50% of all currently used drugs and therefore information on ligand-protein interaction and protein structure are of high interest. At present, data for only one GPCR, bovine rhodopsin in the ground state, are available. The first challenge in achieving structural information of integral membrane proteins is to produce large amounts of active protein.
We performed protein expression in *Escherichia coli* as inclusion bodies using high-density-fermentation. The inclusion bodies were solubilized in the ionic detergent SDS and then the soluble protein was purified using immobilized metal ion affinity chromatography. For the cholecystokinin type A receptor we obtained more than 500 mg of inactive protein from one 8 l fermentation.
The folding of the receptor is carried out *in vitro* by fast dilution of soluble protein in a buffer system containing non denaturating detergents. Above a critical concentration these detergents form micelles, which keep the membrane protein in solution. To determine the functionality of the receptor we use fluorescence measurements were the cholecystokinin octapeptide is titrated to the receptor in the micelle state. In first results we were able to determine a K_D value for the cholecystokinin type A receptor of around 100 nM.

P-184

Novel Viral Activation of a Receptor Tyrosine Kinase

J. E. Oates, A. M. Dixon
Department of Chemistry, University of Warwick, Coventry, UK

The E5 oncoprotein from Bovine Papilloma Virus binds to and activates the platelet-derived growth factor beta receptor (PDGFβR), its cellular target, via a novel mechanism. Unlike the natural ligand (PDGF), E5 binds to the receptor's transmembrane (TM) region leading to active PDGFβ receptor dimer formation, transphosphorylation of SH2 domains and activation of downstream signalling. E5 is a 44 kDa hydrophobic protein comprised almost entirely of a single TM helix. Many studies have shown that E5 forms homodimers that bind PDGFβR strongly and selectively in order to activate the receptor.
The importance of the TM domains in mediating this association has lead to focussed mutagenesis of these regions and identification of protein-protein contacts within both the E5 homodimer and the E5-PDGFβ receptor complex. We have investigated in more detail the functional and structural importance of individual residues and sequence motifs in the TM domains of both proteins in order to refine the current, highly simplistic model of activation.
To investigate interactions between the TM domains, peptides corresponding to the TM domains of E5 and PDGFβ receptor have been examined using a range of biochemical and biophysical techniques to probe their oligomeric state, secondary structure, and the energetics of association in vitro. Oligomersiation has also been investigated *in vivo* in natural membrane bilayers, and preliminary structural investigations are also underway. Results so far shed new light on E5 homodimer formation, and suggest a possible alternative mechanism to PDGFβ receptor activation.

P-185

The use of fluorinated and hemifluorinated surfactants in cell-free synthesis of membrane proteins

K.-H. Park[1], C. Berrier[2], F. Lebaupain[1], B. Pucci[3], J.-L. Popot[1], A. Ghazi[2], F. Zito[1]
[1]Institut de Biologie Physico-Chimique, UMR 7099, CNRS et Université Paris-7, Paris, France, [2]Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, UMR 8619, CNRS et Université Paris-Sud, Orsay, France, [3]Laboratoire de Chimie Bioorganique et des Systèmes Moléculaires Vectoriels, Université d´Avignon et des Pays du Vaucluse, Avignon, France

Perfluorinated and hemifluorinated surfactants are lipophobic and, as such, non-detergent. While they do not solubilize biological membranes, they can, after conventional solubilization, substitute to detergents to keep membrane proteins soluble, which generally improves their stability (Breyton *et al.* FEBS Lett. 2004. 564:312-8). We have previously demonstrated that functional membrane proteins can be synthesized *in vitro* in the absence of membrane but in the presence of detergents (Berrier *et al.* Biochemistry 2004. 43:12585-91). Here, we show that fluorinated surfactants can be used directly for *in vitro* synthesis of membrane proteins: they do not interfere with protein synthesis even in high concentrations, which is often not the case for most detergents, and they provide a suitable environment for MscL, a pentameric mechanosensitive channel, to fold and oligomerize to its native, functional state. Moreover, following synthesis, per- and hemifluorinated surfactants can be used to deliver directly MscL to preformed lipid vesicles.

O-186

Folding and assembly of light-harvesting chlorophyll a/b protein: Fluorescence and EPR kinetics

H. Paulsen
Institut für Allgemeine Botanik, Johannes-Gutenberg-Univ. Mainz, Germany

The major light-harvesting chlorophyll *a/b* protein (LHCIIb) of the photosynthetic apparatus in higher plants is the most abundant membrane protein in plants. During its biogenesis, the apoprotein of about 25 kDa is assembled with 14 chlorophyll and 4 carotenoid molecules; the mechanism of LHCIIb formation is unknown. The LHCIIb apoprotein spontaneously folds and assembles pigments in detergent solution. Time-resolved fluorescence measurements of this process revealed that chlorophyll *a* binds in a faster step (10 s to 1 min) whereas most of chlorophyll *b* binds more slowly (1 to several min) leading to an intermediate complex containing chlorophyll *a* but little or no chlorophyll *b*. The resulting two-step model of LHCIIb assembly is able to explain why the chlorophyll *a:b* stoichiometry in LHCIIb is well defined although several chlorophyll binding sites are known to be able to bind either chlorophyll.
Secondary structure formation in the apoprotein appears to be coupled with pigment binding, as the α helix content increases following the same two-phase kinetics. To analyse other folding events such as 3D structure formation of the hydrophilic protein domains and the juxtaposition of the α helices, we have used pulsed electron paramagnetic resonance (EPR) which allows to measure distances between spin labels attached to the LHCIIb protein. By combining EPR with a freeze-flow setup, we have measured the changes in intramolecular distances during LHCIIb assembly in the time range of ms to min. In principle, this technique should be able to yield a full 3D description of the protein folding process.

Abstracts

– Membrane protein folding –

P-187

Interaction of Equinatoxin II with membranes: why is the presence of sphingomyeline crucial?

D. Poger, A. E. Mark
MD Group, School of Molecular and Microbial Sciences, The University of Queensland Brisbane QLD 4067, Australia

Equinatoxin II (EqtII) is a lethal toxin produced by the sea anemone Actinia equina L. It is a member of the actinoporin family. Actinoporins induce the formation of pores in the plasma membrane via a multistep pathway that includes membrane binding, conformational change and oligomerization. However, the precise details of membrane binding, insertion and formation of a functional pore are unknown. Experimental evidence suggests that the N-terminal region and a surface aromatic cluster embed first in the membrane, following which there is an irreversible conformational change within the peptide leading to the formation of pores. Whereas the initial binding to the membrane is relatively lipid insensitive, pore formation requires the presence of sphingomyeline (SM).
Atomistic molecular dynamics simulations of EqtII in the presence of model membranes have been performed with the aim of identifying key residues involved in membrane binding and the insertion, as well as the nature of the interaction with lipids. By varying the bilayer composition (phosphatidylcholine (PC)-only or SM-containing lipid bilayers), we have worked toward the isolation of the different stages of the process of pore formation. For example, the initial interaction phase has been investigated using a PC-membrane to which EqtII binds but does not induce pores. In simulations involving PC/SM bilayers, larger structural rearrangements are expected. These simulations will provide crucial structural and mechanistic details of pore formation at an atomic scale.

P-188

Molecular determinants of KcsA clustering: the role of cytoplasmatic domains

M. L. Renart, A. Fernandez, M. L. Molina, J. A. Poveda, J. A. Encinar, F. N. Barrera, J. M. González-Ros
Instituto de Biologia Molecular y Celular, Universidad Miguel Hernandez, Elche, Spain.

The potassium channel KcsA is an integral membrane protein from *S.lividans*, used as a model system for studies on ion channel and oligomeric membrane proteins. It can be easily purified and its atomic structure had been solved by X-ray diffraction. KcsA is formed by the assembly of four identical subunits around a central aqueous pore. Each subunit contains 2 transmembrane segments and two cytoplasmatic domains, the N-terminal and the C-terminal segments. Its secondary structure is predominantly alpha-helical and it is resistant to denaturing agents like 8 M urea, 6 M guanidinium chloride or 2% SDS.
Using the solubilized form of KcsA (in β-D-dodecylmaltoside) we found that protein tetramers can organize themselves into supramolecular assemblies in a concentration dependent fashion. Such clustering occurs more markedly when reconstituting the protein into lipid vesicles and indeed, it has also been detected in vivo. Moreover, the heterogeneity of the resulting clusters have been invoked to explain functional diversity. Here, we present evidence to support that putative molecular determinants for the clustering process are found within the N-terminal end of the channel protein.

O-189

Functional characterization of human neuropeptide Y receptors expressed in *E. coli*

P. Schmidt[1], D. Lindner[2], S. Schimmer[1], C. Montag[1], R. Rudolph[1], A. G. Beck-Sickinger[2], D. Huster[1]
[1]Institute of Biotechnology, MLU Halle-Wittenberg, Germany, [2]Institute of Biochemistry, University Leipzig, Germany

Human neuropeptide Y receptors are integral membrane proteins and belong to the G protein-coupled receptor family (GPCRs). The understanding of the ligand-protein interaction as well as of the entire protein structure is a prerequisite for the successful production of receptor-active drugs and is therefore of high interest for pharmaceutical research.
To obtain milligram quantities of protein the receptor was overexpressed in *E. coli* as inclusion bodies. The refolding *in vitro* takes place in the presents of non-denaturating detergents well above the critical micelle concentration, so that the folded receptors are kept in solution by micelles.
In this micelle state we performed affinity assays, such as fluorescence measurements and competitive ligand binding assays with ^{3}H-labeled NPY, the ligand for the Y receptors, as tracer. The radiolabeled ligand binding assay was adapted to the application of micelles and used to confirm specific binding in comparison with active Y receptors expressed in mammalian cells. The competition experiments showed an IC_{50}value of around 40 nM for Y1-receptors in micelles. With fluorescence measurements we were able to show quenching in the ligand-receptor complex of up to 15% of the total fluorescence signal. This technique was also used in titration experiments with NPY to determine ligand affinity for the receptors with K_D values of around 28 nM for the Y1 and 3 nM for the Y2 receptor, respectively.

P-190

Role of local internal viscosity in protein dynamics

Z. Simon, J. Toth, P. Medveczky, L. Gombos, Z. Yang, B. Jelinek, L. Szilagyi, L. Gráf, A. Málnási-Csizmadia
Department of Biochemistry, Institute of Biology, Eotvos Lorand University, Pazmany Peter setany 1/C, H-1117 Budapest, Hungary

Upon activation of trypsinogen four peptide segments flanked by hinge glycine residues undergo conformational changes. To test whether the degree of conformational freedom of hinge regions affects the rate of activation we introduced amino acid side chains of different characters at one of the hinges (position 193) and studied their effects on the rate constant of the conformational change. This structural rearrangement leading to activation was triggered by a pH-jump and monitored by intrinsic fluorescence change in the stopped-flow apparatus. We found that an increase in the size of the side chain at position 193 is associated with the decrease of the reaction rate constant. To analyze the thermodynamics of the reaction, temperature dependence of the reaction rate constants was examined in a wide temperature range (5-60°C) using a novel temperature-jump/stopped-flow apparatus developed in our laboratory. Our data show that the mutations do not affect the activation energy (the exponential term) of the reaction, but they significantly alter the preexponential term of the Arrhenius equation. The effect of solvent viscosity on the rate constants of the conformational change during activation of the wild type enzyme and two mutants was determined and evaluated on the basis of Kramers' theory. We also determined internal friction parameters of the conformational change of the mutants and measured its temperature dependencies. There is an exponential relation between internal friction and temperature. We propose that the reaction rate of this conformational change is regulated by the internal molecular friction which can be specifically modulated by mutagenesis.

Abstracts

– Membrane protein folding –

P-191

Oligomeric behavior of two RND transporters in micellar solution of detergent

D. Stroebel[1], V. Sendra[1], C. Ebel[1], D. Cannella[1], K. Helbig[2], D. H. Nies[2], J. Coves[1]
[1]Institut de Biologie Structurale, CNRS-CEA-UJF, Grenoble, France, [2]Institut für Mikrobiologie, Halle, Germany

We have used analytical ultracentrifugation to explore the oligomeric states of AcrB and CusA in micellar solution of detergent. These two proteins belong to the resistance, nodulation and cell division (RND) family of efflux proteins that are involved in multiple drug and heavy metal resistance. Only the structure of AcrB has been determined so far. Although functional RND proteins should assemble as trimers as AcrB does, both AcrB and CusA form a mixture of quaternary structures (from monomer to heavy oligomer) in detergent solution. The distribution of the oligomeric states was studied as a function of different parameters: nature and concentration of the detergent, ionic strength, pH, protein concentration. This pseudo-heterogeneity does not hamper the crystallization of AcrB as a homotrimer.

O-192

Comparison of transmembrane and loop contributions to stabilization and folding of membrane proteins

O. Tastan, J. Wu, N. Yanamala, J. Klein-Seetharaman
Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, U.S.A.

In order to develop a better mechanistic understanding of membrane protein folding, we investigated the predicted (un)folding pathways of alpha-helical membrane protein structures. An in-depth analysis of all available rhodopsin structures using FIRST software [1] revealed that mammalian rhodopsin structures have a stability core that is characterized by long-range interactions comprising positions from both extracellular loop and transmembrane regions, while those of bacteriorhodopsin are dominated by interactions within individual and groups of helices, consistent with the two-stage hypothesis [2]. These results support the conclusion that although the two-stage model to some extent explains the mechanisms of folding and stability of bacteriorhodopsin; it fails to account for the folding and stability of rhodopsin for which we propose a long-range loop and helix interactions model of folding. Additional support for this model comes from studies using Fast Contact 1.0 software [3], which show that the second extracellular loop in rhodopsin plays a particularly important role in its stabilization. Analysis of all available alpha-helical transmembrane protein structures with resolution better than 3Å using FIRST suggests that there is a continuum between the contributions of two-stage and long-range interactions in the folding of membrane proteins.
References: [1] http://flexweb.asu.edu/software/first/ [2] Tastan et al. (2007) Photochem. Photobiol., in press. [3] Camacho & Zhang (2005) Bioinformatics 21, 2534-6.

P-193

Folding simulations of membrane proteins in implicit generalized Born membranes

J. P. Ulmschneider[2], M. B. Ulmschneider[1]
[1]Univ. of Oxford, U.K., [2]Univ. of Rome ´La Sapienza´, Italy

We investigate the temperature dependent folding of two transmembrane helical peptides: The membrane spanning segment 2 of the acetylcholine receptor from Rattus norvegicus and the membrane spanning segment of human glycophorin A. The replica exchange method was combined with a recently developed implicit membrane representation based on the generalized Born theory of solvation. The protein was simulated using the OPLS all-atom force field in an efficient Monte Carlo setup. A previously developed concerted rotation backbone move algorithm facilitates the efficient folding of the protein. This method has been demonstrated to perform equally well, if not slightly better when compared to molecular dynamics sampling in protein folding simulations. In the present study the peptides fold from an extended structure into native-state trans-membrane helices within 300-500 million Monte Carlo moves, corresponding to a couple of days on a 10 CPU PC cluster. Further simulation to several billion Monte Carlo steps demonstrate the helices to remain stable and integrated into the membrane. While the lower temperature replicas fold into native or near-native helical structures the higher temperature replicas retain an unfolded conformation. To verify that the computational setup is free of a helical bias an alanine-rich peptide of known experimental helicity was studied in 10 billion step replica exchange Monte Carlo simulations, equivalent to a combined $\sim$10 μs of molecular dynamics. The result demonstrates that the model is free of a helical bias, an important requirement for the successful modeling of helical membrane spanning peptides.

P-194

Atomic detail membrane protein folding studies using an implicit generalized Born membrane

M. B. Ulmschneider[1], J. P. Ulmschneider[2]
[1]Univ. of Oxford, U.K., [2]Univ. of Rome ´La Sapienza´, Italy

An implicit membrane representation based on the generalized Born theory of solvation has been developed. The method was compared against experimental data from translocon inserted hydrophobic polypeptides and validated by comparison with an independent dataset of 6 membrane associated peptides and 8 integral membrane proteins of known structure and orientation.
All proteins investigated orient and insert correctly into the membrane. Remarkably the model correctly predicts a partially inserted configuration for the monotopic membrane protein Cyclooxygenase, matching experimental and theoretical predictions.
The membrane model is applied to the folding of two transmembrane helices, M2 from influenza A and the membrane spanning segment of virus protein U from HIV-1. Replica exchange simulations employing the OPLS all-atom forcefield were used. Conformations are sampled with a concerted rotation Monte Carlo method for backbone moves and standard Monte Carlo for side chain moves. This method has been demonstrated to perform well when compared to molecular dynamics sampling. Both peptides fold from an extended structure into native-state trans-membrane helices within 500 million Monte Carlo moves, corresponding to $\sim$3 days on a 10 CPU PC cluster. Further simulation to 2 billion Monte Carlo steps demonstrate the helices to remain stable and integrated into the membrane. While the lower temperature replicas fold into native or near-native helical structures the higher temperature replicas retain an unfolded conformation, suggesting the underlying model is free of a helical bias.

Abstracts

– Membrane protein folding –

P-195

Structural and biophysical characterization of solubilized apolipoprotein B-100

I. Waldner, R. Prassl
Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, Graz, Austria

Apolipoprotein B-100 (apoB-100), the single protein moiety of human low density lipoprotein (LDL), is a large amphipathic glycoprotein which plays a triggering role in the progression of atherosclerosis. Nevertheless, detailed knowledge on its structure is still minimal. In this context, we subjected a set of differentially solubilized apoB-100 preparations to various morphologically studies, to clarify its structural organization. Recently, we were able to restore a 3-D low-resolution model of the apo-B100 delipidated by the non-ionic detergent Nonidet P-40 (NP-40) from small-angle neutron scattering data. Most interestingly, the protein adopts an elongated shape, consisting of distinct subdomains connected by flexible regions. In contrast, lipid-associated apoB-100 is more densely wrapped around the surface of spherical LDL particles. A relatively new approach for solubilization of membrane-associated proteins is the use of small amphiphilic peptides with a structure similar to that of phospholipids, as well as short-chained, amphiphilic polymers. We currently subject apoB-100, stabilized in these detergent-like surfactants, to different methods such as circular dichroism or HPLC-MS, proving that the surfactants were capable of fully stabilizing the protein in solution. Neither amphiphilic polymers nor lipid-like peptides altered the secondary structure significantly, compared to native apoB-100 on LDL or NP-40 solubilized apoB-100. Thus, these molecules appear to constitute very promising new classes of surfactants for studies of apoB-100, besides classical detergents. Further studies concerning this matter are in progress.

P-196

Ab initio folding simulations and atom level structure predication of the decapeptide Chignolin

J. Wang, X. Dou
The key biophysics lab of Shandong, De zhou, Shandong, China

Chignolin is an 10 residue folded peptide. The folding free energy landscape of the peptide has been explored based on MD. The results show that the peptide is generally a stable downhill folder sliding from the fully extended state into the folded state. But there is a short-lived intermediate state if folding starting from the initial right α helix. Although the peptide folds starting from different initial states they converge at similar folded structures. The backbone RMSD of the lowest free energy structures from the NMR native structure of Chignolin is only 0.114 nm.

Abstracts

– Multiscale simulations –

O-197

Mechanical modelling of the cellular propulsion.

J.-M. Allain[1], I. Kuznetsov[2], M. Herant[2], M. Dembo[2]
[1]LMS, Ecole Polytechnique, 91128 Palaiseau, France, [2]Departement of biomedical engineering, Boston University, Boston MA 02215, USA

The ability of the living cells to move by themselves is a fascinating property, at least from a mechanical point of view. This motion uses a controlled reorganization of the cellular cytoskeleton. In particular, the actin filaments grow mainly at the leading edge of the cell, creating a network which pushes the membrane and allows the motion of the cell. In the last few years, the biochemistries of the actin polymerisation and of the network formation have been studied intensively, and we have now-a-day a good idea of the mechanisms. However, the associated mechanical properties are considerably less understood. A reason may be that they involve many different players: the actin organisation, its interaction with the cell membrane or the adhesion between the cell and the external medium.
A first step to explore these mechanical properties is to study a model system, the bacteria Listeria. This bacterium is able to move inside of an infected cell by using the surrounding actin. To do that, the bacterium polymerises of the actin filaments at its back. There are now-a-day many experiments on Listeria or on mimetic systems. They have elucidated the mechanisms behind this propulsion, at least qualitatively.
We present a numerical model of actin behaviour, taking into account the surrounding aqueous medium. This model allows us to reproduce experimental results on Listeria propulsion, such as actin density in bacteria tail or bacteria speed. We discuss how this model has to be extended in order to achieve a more complete description of the Listeria propulsion, or to be adapted to the cellular motility.

P-198

Using coarsed-grained models to understand differences in folding behaviour of homologous proteins.

L. R. Allen, E. Paci
Department of Physics and Astronomy, University of Leeds, UK.

The folding mechanisms of proteins with different sequences and highly homologous structures provide a simple, comparative, picture of complex folding mechanisms, and have been the object of thorough experimental investigation. Here we report the analysis of long equilibrium simulations of a simple, structure-based protein model, in which many folding and unfolding events are recorded. The results show that small differences in interatomic interactions can affect the overall thermodynamic and kinetic properties of the protein. Moreover, the kinetic properties of the immunity proteins Im7 and Im9 calculated from simulation data show surprising agreement with experiment. In particular, Im7 shows the presence of an intermediate which is populated at the folding temperature while Im9 has a sharp transition with no significantly populated intermediate. The structural characterisation of the Im7 intermediate suggests mutants of Im9 which fold via three-state mechanisms.

P-199

Molecular dynamics study of the reaction mechanism of Tim44 protein with lipids

Y. Avneon, M. Gutman, E. Nachliel, A. Azem, Y. Tsfadia
Department of Biochemistry, Tel Aviv University, Israel

The final step of the translocation of proteins across the mitochondrial membrane is mediated by a protein motor that at its heart stands the matrix chaperone mHsp70. The Tim44 protein is suggested to function as adaptor that anchors mHsp70 to the TIM23 complex motor. It is known that Tim44 interacts with acidic membranes through its C terminal domain [1]. The precise mechanism of the interaction is unknown.
In the present communication we report the primary results of a molecular dynamics study of the Tim44 interaction with fatty acids based on the crystal structures of the C terminal domain of Tim44, as determined for the Yeast (PDB code: 2FXT) and the Human (PDB code: 2CW9) proteins. Both structures share a common feature, a hydrophobic cavity large enough to accommodate a fatty acid molecule [2]. Our results describe the trajectories and the energetic profiles of two types of interactions of Tim44 with soluble fatty acid molecules: (a) The passage of a fatty acid molecule, all the way from the bulk into the hydrophobic pocket of the protein that is suspected for anchoring it to the membrane; (b) The binding of palmitate anions with the positively charged, highly conserved α-helix at the N-terminus end of the C terminal domain of Tim44.
[1] Weiss, C., et al., Proc Natl Acad Sci U S A, 1999. **96**(16): p. 8890-4.
[2] Josyula, R., et al., J Mol Biol, 2006. **359**(3): p. 798-804.

P-200

The influence of interdomain interactions on intradomain motions in yeast phosphoglycerate kinase

E. Balog, Z. Palmai, J. Fidy
Department of Biophysics and Radiation Biology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Molecular dynamics simulation was performed to examine the inter- and intradomain motions of the two-domain enzyme yeast phosphoglycerate kinase without the presence of substrates. To elucidate contributions from individual domains, simulations were carried out on the complete enzyme as well as on each isolated domain. The enzyme is known to undergo a hinge-bending type of motion as it cycles from an open to a closed conformation to allow the phosphoryl transfer occur. Analysis of the correlation of atomic movements during the simulations confirms hinge bending in the nanosecond timescale: the two domains of the complete enzyme exhibit rigid body motions anticorrelated with respect to each other. The correlation of the intradomain motions of both domains converges, yielding a distinct correlation map in the enzyme. In the isolated domain simulations—in which interdomain interactions cannot occur—the correlation of domain motions no longer converges and shows a very small correlation during the same simulation time. This result points to the importance of interdomain contacts in the overall dynamics of the protein.

Abstracts

– Multiscale simulations –

P-201

Monolayer-bilayer transformations of lung surfactant from molecular dynamics simulations

S. Baoukina[1], L. Monticelli[1], M. Amrein[2], D. P. Tieleman[1]
[1]Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, AB, Canada, [2]Department of Cell Biology and Anatomy, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

The aqueous lining of the lung exposed to the air is covered by a mixed lipid-protein film of lung surfactant. The main function of lung surfactant is to reduce the surface tension of the air-water interface to low values. This function requires the exchange of material between lipid monolayer at the interface and lipid reservoirs in the aqueous lining under dynamic compression and expansion of the interface during the breathing cycle. We simulated reversible exchange of material between the monolayer and lipid reservoirs under compression and expansion of the interface using a mixture of DPPC, POPG, cholesterol and surfactant-associated protein C (SPC) as a functional analogue of mammalian surfactant. The simulations show that the bilayer aggregates are unstable in the air sub-phase and stable in the water sub-phase. The monolayer collapses into the water sub-phase upon compression and forms bilayer folds. Upon monolayer re-expansion, the material is transferred from the folds back to the interface. The simulations also show that the connectivity of the bilayer aggregates in the water sub-phase to the monolayer is necessary for the reversibility of monolayer-bilayer transformation.

P-202

Energetics of ion permeation in ion channels from free energy simulations

T. Bastug, S. Kuyucak
School of Physics, University of Sydney, NSW 2006, Australia

Predicting the functional properties of an ion channel from its structure is the ultimate goal of research in ion channels. Molecular dynamics (MD) simulations provide the best tool for this purpose, but unfortunately they are too slow for estimating conductance of a channel directly. An indirect route that has become feasible in recent years is to calculate the energy profiles of permeating ions from free energy simulations and employ these in a coarse-grained method such as Brownian dynamics to estimate the conductance. Here we discuss application of this methodology to two well known ion channels: gramicidin A and potassium. We construct the potential of mean force for an ion along the permeation pathway in each case and discuss its implications for conductance from Brownian dynamics simulations. Especially in gramicidin A, some discrepancies are found, e.g. larger than expected energy barriers and shallower binding sites. The most likely source of these discrepancies is the neglect of the polarization interaction in the current MD force fields. Thus our results provide a motivation for including the polarization interaction in the next generation of force fields. Convergence and consistency of the results are checked by comparing four different free energy methods: Umbrella sampling with weighted histogram analysis, free energy perturbation, thermodynamic integration and steered MD with Jarzynski's equality [1]. We find that the first three methods are consistent with each other but not the last one, which appears to suffer from equilibration problems.

[1] T. Bastug and S. Kuyucak, Biophys. J. 90 (2006) 3941-3950.

O-203

The molecular mechanisms of protein folding and signal transduction revealed by multiscale modelling

P. G. Bolhuis
van´t Hoff institute for Molecular Sciences, University of Amsterdam, The Netherlands

All-atom explicit solvent molecular dynamics (MD) simulations of protein folding and other conformational changes occurring in signal transduction remain a great challenge in computational biophysics because of the long time scales involved. These long times are due to high free energy barriers between stable states. We apply recent methods to tackle these barriers, including Replica Exchange (REMD) and Metadynamics, to the bacterial sensor Photoactive Yellow Protein (PYP). PYP signals the presence of blue light by undergoing a photo-cycle, a series of conformational changes starting with a fast trans-cis isomerisation of the chromophore, followed by a proton transfer from Glu46 to the chromophore. We study this crucial reaction using CPMD QM/MM combined with Metadynamics. The protein subsequently partially unfolds to the signalling state. Employing REMD we explore the formation of the signalling state and the refolding to the receptor state.

While these simulations allow a thermodynamic analysis they do not preserve the dynamical pathways that are needed to gain insight into the kinetics and mechanism of conformational change. In contrast, Transition Path Sampling (TPS) is a technique designed for harvesting an ensemble of dynamical transition paths between two stable states separated by high free energy barriers. We use TPS to collect unbiased folding pathways for the trp-cage mini-protein as well as for several beta hairpins with experimental folding times of the order of microseconds. The path ensemble is subsequently analysed for reaction coordinates and rates. These results can then be further used to improve coarse grained Markovian State Models.

P-204

Malleability of cytochrome P450 3A4 from a theoretical perspective

D. Bouvier[1], V. Hamon[2], L. Perrin[2], F. André[2]
[1]ABI, Université Paris VI, 12, rue Cuvier, F-75005, France, [2]CEA, iBiTec-S, URA2096 CNRS, Gif-sur-Yvette, F-91191, France

Understanding, at a molecular level, the recognition mechanisms of substrates by human cytochrome P450 3A4 remains a holly grail for both pharmaceutical industries and academics. Indeed, this hemoprotein has peculiar enzymologic features. It is both multisubstrate, multispecific and exhibits homo and heterotropic cooperative effects. Practically, this enzyme is involved in the metabolism of 50% of market drugs. Several models have been proposed to explain this non canonical behaviour, some imply a large malleability of the enzyme. Assessing experimentally the malleability of an enzyme is not trivial, molecular simulation is therefore a method of choice to address this issue. Normal Mode Analysis and Principal Component Analysis of molecular dynamics trajectories can give fruitful information with respect to the global motion of a protein and its deformability. These two types of studies and analysis have been applied to 3A4. Analysis of deformation energies associated with the displacement of atoms in mode(s) revealed that 3A4 is highly deformable compared to other proteins (eg myoglobin). There is a continuous gradient of flexibility which decreases from the inner core to the outer region of the protein. Interestingly, areas that exhibit highest flexibility correspond to the substrate recognition sequences. Additionally, PCA of a linear trajectory of 14ns or 7 parallel trajectories of 2ns show that protein evolved in a wide and floppy conformational space. Analysis of trajectories and comparison with NMA suggest that MD simulation is not a method of choice for studying the conformational behaviour of 3A4.

Abstracts

– Multiscale simulations –

P-205

Water percolation governs polymorphic transition and conductivity of DNA

I. Brovchenko[1], A. Krukau[1], A. Oleinikova[1], A. K. Mazur[2]
[1]Physical Chemistry, Dortmund UniVersity, Otto-Hahn-Str. 6, Dortmund D-44227, Germany, [2]Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, Paris 75005, France

We report on the first computer simulation studies of the percolation transition of water at the surface of the DNA double helix. At low hydrations, only small water clusters are attached to the DNA surface, whereas, at high hydrations, it is homogeneously covered by a spanning water network. The spanning water network is formed via a percolation transition at an intermediate hydration number of about 15 water molecules per nucleotide, which is very close to the midpoint of polymorphic transitions between A- and B-forms of the double helix. The percolation transition can occur in both A- and B-DNA hydration shells with nearly identical percolation thresholds. However, the mechanism of the percolation transition in A- and B-DNA is qualitatively different in regard to the roles played by the two opposite grooves of the double helix. Free ions can shift the percolation threshold by preventing some water molecules from hydrogen bond networking. Formation of spanning water networks results in sigmoid like acceleration of long-range ion transport in good agreement with experiment.

1. Brovchenko, I.; Krukau, A.; Oleinikova, A.; Mazur, A. K. *Phys.Rev. Lett.* **2006**, *97* (13), 137801(4).
2. Brovchenko, I.; Krukau, A.; Oleinikova, A.; Mazur, A. K. *J. Phys. Chem. B,* **111** (12), 3258 -3266, 2007.

P-206

Biomechanical quality of normal and osteoporotic bones: experimental and finite element analysis

U. Comelekoglu[1], S. Korkutan[1], H. Mutlu[2], A. Comelekoglu[3], S. Bagis[1], A. Yildiz[1], S. Yalin[4]
[1]MEU, Medical Faculty, Mersin Turkey, [2]MEU, Machine Engineering, Mersin, Turkey, [3]MEU, Technical Sciences, Mersin, Turkey, [4]MEU, Pharmacy Faculty, Mersin, Turkey

14 young adult female rats used in the study. Rats were divided into two groups of 7, as the control group and the ovariectomized (OVX) group. Rats in the OVX group went under bilateral ovariectomy following a ventral incision. Bone mineral density was measured by dual energy X-ray absorbsiometry. 14 weeks after the ovariectomy operation, femurs of all the rats in both groups were taken out and underwent biomechanical tension tests to determine deformation, stress and strain values. In order to carry out finite element analysis, Tomography was used to obtain lateral cross-sections of each bone and this data was used to create 3D computerized models of the bones. ANYSIS 9.0 software was used in the finite element analysis to attain deformation, stress and strain values. Both in normal and osteoporotic bones, deformation, stress and strain values obtained through biomechanical experiments and finite element analysis proved to be comparable ($p>0.05$). As a clinical application of the knowledge we gained from this experiment, we suggest that FEM analysis can be used in vivo to determine the bone biomechanical quality of osteoporotical patients.

P-207

Insight into potassium channel gating using metadynamics

C. Domene
Physical & Theoretical Chemistry Lab, University of Oxford, U.K.

Despite the success and deep influence that molecular dynamics simulations have had on ion channel research, they suffer from limitations that reduce the scope of their applications. One of these severe constraints is the limited time scale that current computers and sampling algorithms explore. Simulation times are generally too short to yield proper sampling of conformational changes of biomolecules and it is difficult to extract functionally relevant motions. Recently, there have been a number of exciting developments aimed at extending both the time- and length-scales accessible to biophysical simulations. Metadynamics is one of these methods. Some applications to ion channel gating will be presented.

P-208

Molecular dynamics simulations of the dihexanoyl phosphatidylcholine detergent.

J. P. Duneau, S. Ranaldi, J. Arce Lopera, J. N. Sturgis
Université de la Méditerranée LISM/CNRS Marseille, France

Short chain lecithins form a particular class of amphiphiles which are widely used in biochemical studies of membrane proteins (MP). Among them the Dihexanoyl phosphatidylcholine detergent (DiC6PC) has been successfully used in the structure elucidation of MP by Xray-crystallography, and NMR. DiC6PC is a bicatenary phospholipid with a relatively high CMC (14 mM) and a low aggregation number (20 molecules). Neutron diffraction studies have shown very mono disperse solutions over a wide range of detergent concentration. However, these properties may appear to conflict with the molecular view of phosphatydylcholine lipids organisation within the membrane and particularly with the planar arrangement of the head groups in the bilayer. Thus, a hypothesis is that the experimental behaviour of Di6PC solutions should emerge from the details of their molecular interactions and structures. To test this, we make use of molecular dynamics simulations to follow the spontaneous aggregation of DiC6PC monomers on the time scale of 10 ns. Also we have studied the behaviour of various aggregates with size constrained above or over the experimental aggregation number. An energetic analysis shows that the driving force of the aggregation is the interactions between water molecules. It was observed that detergent phosphocholine headgroups are still maintained in planar sheet structures that preclude the formation of isotropic micelles. Around the aggregation number, bilayered micelles are favoured, giving rise to ellipsoid structures as proposed by neutron diffraction data.

Abstracts

– Multiscale simulations –

O-209

Coarse grained modelling of membrane systems

J. W. Essex[1], M. Orsi[1], J. Michel[1], W. Sanderson[2]
[1]School of Chemistry, University of Southampton, Southampton, United Kingdom, [2]Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium

Classical atom-based computer simulations are computationally demanding and therefore restricted in the length and time scales to which they may be applied. However, many properties of biologically relevant membranes require larger simulation systems run for longer times than may conveniently be accessed using conventional atom-based models. To address this deficiency, coarse grained representations of lipid molecules have been developed in which groups of atoms, and their associated intermolecular forces, are subsumed into single interaction sites. A range of models exist with different levels of granularity, and consequently different ranges of applicability. In this presentation, existing coarse grained membrane models will be briefly reviewed. A new membrane model based on a combination of Lennard Jones and anisotropic Gay Berne potentials will then be presented. Unlike many coarse-grain models, electrostatic interactions are fully retained through inclusion of dipoles and charges. Water is modelled by the soft sticky dipole potential. Starting from a random solution, lipids spontaneously self-assemble into a bilayer that reproduces quantitatively all major experimentally-determined physical properties of membranes. Furthermore, the potentials employed are compatible with atomistic force fields. Applications to drug permeability studies and how this approach may be extended to model other components of biological membranes will be discussed.

P-210

Hydrophobic effect and dissociation of benzene aggregates in water under high hydrostatic pressure

A. D. S. Gonçalves[1], E. R. Caffarena[2], F. M. C. Vieira[3], P. G. Pascutti[1]
[1]UFRJ – IBCCF, Brazil, [2]FIOCRUZ, Brazil, [3]UnB, Brazil

Water forms organized structures around polar and apolar molecules and in some critical conditions (pressure, temperature, etc.), water can surround hydrophobic molecules becoming a powerful solvent. Objectives: To discuss the pressure ¨critical effects¨ we carried out molecular dynamics simulations (MDS) of 18 solvated benzene molecules. Methods: Parameters from benzene molecules were described by OPLS force field, atomic charges were computed with HF 6-31G* and CHELPG method. After energy minimization the system with restrained solute heavy atom positions was submitted to 500 ps of MDS followed by six consecutive 5 ns unrestrained MDS at different pressures, ranging from 1 bar to 5 kbar, at 280 K. Volume variation, diffusion coefficient, radial atomic pair distribution functions and the RMSD of benzene molecules were monitored. Results: From our results we can observe that up to 3 kbar the structured second hydration layer is lost and the benzene clusters start to break up gradually. Until 2 kbar, the solubility and diffusion of benzene molecules are conversely proportional to the pressure enhancement. Above 2-3 kbar the behavior is inverted, in such a way that increasing pressure causes solubility enhancement of benzene, which is also confirmed by diffusion coefficient behavior. Conclusion: Water structure around apolar groups takes an important role in hydrophobicity. Changing the physical conditions of the aqueous environment applying high hydrostatic pressure induces changes in the solvation shell that can be the main responsible for enhancing in solubility of apolar molecules.

P-211

On the molecular mechanism of ADP/ATP mitochondrial carrier tranport: insights from principal components analysis of MD simulations.

A. Grottesi[1], D. Di Marino[2], G. Chillemi[1], M. Falconi[2], L. Palmieri[3], A. Desideri[2]
[1]CASPUR, Rome, Italy, [2]Department of Biology, University of Rome “Tor Vergata" and CIBB, Rome, Italy, [3]Department of Pharmaco-Biology, University of Bari and CNR Institute of Bioenergetics and Biomembranes, Bari, Italy

The mitochondrial adenosine diphosphate/adenosine triphosphate (ADP/ATP) carrier has been crystallized in complex with its specific inhibitor carboxyatractyloside (CATR). In the crystal structure, the six-transmembrane helix bundle that defines the nucleotide translocation pathway is closed on the matrix side due to sharp kinks in the odd-numbered helices. The structural dynamics of ADP/ATP mitochondrial carrier is studied by molecular dynamics (MD) simulations of the monomeric form embedded in a POPC lipid bilayer, without the inhibitor. Essential Dynamics has been used to investigated the principal comoponents of Cα atoms positional fluctuations. The calculations show that protein's dynamics sampled on a 20 ns timescale is characterized by the presence of a concerted motion involving odd-numbered helices H1, H3 and H5, using three conserved prolines as molecular hinge, as well as matrix loops linking helices 1,2, 3,4 and 5,6. This finding is consistent with the transposition mechanism proposed to explain nucleotide transposition across inner mitochondrial membrane and suggests a crucial role of H1 and H3 helices motion (as well as of proline residues in the conserved signature sequence) in the conformational change responsible for nucleotide transfer across inner mitochondrial membrane.

P-212

An objective evaluation of information loss in amblyopic perception

A. Iftime[2], A. Thiel[3], R. Sireteanu[1]
[1]Neurophysiology Dept, Max Planck Institute for Brain Research, Frankfurt Germany, [2]Biophysical Dept, UMF “Carol Davila” Bucharest Romania, [3]Dept. of. Biological Psychology, Institute for Psychology, JW Goethe-University, Frankfurt, Germany

The purpose is the quantitatively investigation through a digital method the spatial and temporal visual misperceptions reported by human amblyopic subjects. Twenty-three subjects with strabismic, anisometropic, or mixed amblyopia were asked to describe and sketch their subjective percept of four different geometrical patterns as seen with the amblyopic eye. The stimuli were: two vertical gratings of spatial frequencies of 0.4 cycles/degree and 1.6 cycles/degree, a checkerboard with 0.4 cycles/degree and a rectangular grid with 3.2 cycles/degree. Based on their descriptions, digital images or movies were generated until validated by the subjects. The images were normalized to five levels of gray (matching perceptual luminance). We have developed an algorithm that calculates Shannon entropy on the images produced by the subjects. The entropy was computed for each image or movie reported by the subjects, and compared with the entropy of the original presented images. For the vast majority of the cases we have observed an increase in overall entropy of the described images, as compared with the presented images. We interpret the observed increase of entropy as a measure of loss of information structure in visual flux of the subjects. We plan to analyze in depth the relationship between the aetiology and the severity of the mispercept and the link between the entropy variations and the temporal distortions (unstable perception).

Abstracts

– Multiscale simulations –

P-213

Monte Carlo study of reversible Michaelis Menten enzymatic reactions in crowded media

A. Isvoran[1], E. Vilaseca[2], J.-L. Garces[3], L. Unipan[4], F. Mas[2]
[1]Department of Chemistry, West University of Timisoara (UVT), Romania, [2]Department of Physical Chemistry, Barcelona University (UB)&TheoreticalChemistry Research Centre (CeRQT) of Scientific Park of Barcelona (PCB), Spain, [3]Department of Chemistry, Lleida University (UdL), Spain, [4]Department of Agriculture, University of Agricultural Sciences of Banat, Timisoara, Romania

A fractal-like approach has been proposed to describe reaction kinetics in crowded media where the classical laws are not accomplished. In this work we use Monte Carlo simulations for studying reversible Michaelis Menten (MM) enzymatic reactions in two-dimensional (2D) and three-dimensional (3D) crowded media. Previous simulations for irreversible MM enzymatic reactions reveal fractal-like kinetics only due to the first bimolecular step and the degree of fractality strongly depends on the topological dimension of the media where reaction occurs, on obstacle and reactants concentrations and mobility and also on their sizes. In reversible MM scheme, there are two bimolecular steps that show fractal kinetics. Applying the law of mass action, we obtain the equilibrium constant that is not time dependent, its value being related to the size and concentration of obstacles and the topological dimension of the medium.

P-214

Analysis of the calcium-dependent activation of the oedema factor EF of anthrax by calmodulin

E. Laine[1], J. Hugenin[2], T. E. Malliavin[1]
[1]Equipe de Bioinformatique Structurale, Institut Pasteur, 28, rue du Dr. Roux, 75015 Paris, France., [2]Programa de Posgraduaçao em Quimica Orgânica, Universidade Federal Fluminense, Outeiro de S. Joao Batista s/n, 24020150, Niteroi, RJ, Brazil.

Among the toxic agents secreted by *Bacillus anthracis*, the Gram-positive bacterium responsible for anthrax, the oedema factor (EF) is an adenylyl cyclase that provokes the accumulation of cyclic AMP in host cells. EF is activated by the ubiquitous protein calmodulin (CaM), involved in many calcium-signalling pathways that regulate crucial biological functions. It consists of two pairs of EF-hands linked by a flexible helix. It was shown in the literature that the formation and stability of the EF-CaM complex depends on the number of calcium ions bound to CaM. We analysed the influence of calcium by molecular dynamics simulations. Three 15-ns simulations of the EF-CaM complex were performed with different levels of calcium. The analysis of the trajectories confirms that the complex with two calcium ions bound to CaM is the most stable and reveals that binding or removing calcium ions from CaM is unfavourable for the interaction with EF. EF requires the C-terminal domain of CaM to be in an open conformation while it blocks the N-terminal domain in a close conformation, reducing its affinity for calcium. Apart from this analysis we used a simplified representation of the system, consisting of seven domains, to perform free-energy calculations on each trajectory. These calculations enabled to identify energetical dependencies between domains. Dependency maps vary according to the level of calcium. They give a representation of the energetical influences at play within the complex.

P-215

MD simlulations of proton transport through a model channel yield effect of hydrophobic confinement

J. Lampe, U. W. Schmitt
MPI for Biophysical Chemistry, Göttingen, Germany

Nanopores are of vital importance for many biological transport processes, like e.g. the passage of protons from the inside of cellular compartments via ion channels. The properties of highly confined molecular or ionic fluids may differ widely from their respective bulk properties [1]. Due to confinement the hydrogen-bonded network in water can be significantly disturbed. This may especially affect the properties of proton transport, which is governed by reorganization of the 3-dimensional hydrogen-bonded network. To study the effect of confinement on the structural and dynamical properties of the migrating proton we employ the MSEVB model[2] in combination with a simplified model nanopore/membrane system. To investigate the influence of confinement, both pore radius and membrane thickness can be systematically varied. This allows us to obtain general insight into the molecular picture of proton migration. Therefore our results will be of relevance for a wide range of biological pore/membrane systems. We use umbrella sampling techniques to explore the free energy profile for proton migration through the nanopore. Additionally we compute an ensemble of reactive trajectories, which are analyzed with respect to the detailed molecular transport mechanism. At large pore radii a spontaneous filling of the initially empty nanopore is observed, and as a consequence only a small free energy barrier for proton translocation is revealed by our simulations. In contrast pores with smaller radii, which show no spontaneous filling with water, significantly inhibit proton migration as seen from the computed free energy profile.
[1] Biophys. J. 80 (1691), [2] J. Chem. Phys. 117 (5839)

P-216

Effect of hydration on enzyme activity and dynamics

M. Lopez[1], R. V. Dunn[2], V. Kurkal-Siebert[3], J. C. Smith[3], R. M. Daniel[1]
[1]University of Waikato, Hamilton, New Zealand, [2]University of Manchester, Manchester, England, [3]Universitaet of Heidelberg, Heidelberg, Germany

To understand the molecular basis of the role of water in protein function, several studies have established a correlation between enzyme activity and hydration level. While a threshold of hydration of 0.2 *h* is usually accepted for the onset of enzyme activity, recent works show that enzyme activity is possible at water contents as low as 0.03 *h* [1]. Since, the pig liver esterase catalyses alcoholysis reaction, in which water is not a substrate, we have investigated activity at zero water content using a gas phase catalytic system. The dry state of the enzyme was defined by quantification of water molecules with ^{18}O-labeled water. In order to correlate the onset of enzyme activity at low hydration with the protein dynamics, we performed neutron scattering and MD simulation experiments. Our preliminary results seem to show a dynamical transition at hydration levels as low as 3%[2].
[1]P.A. Lind, R.M. Daniel, C. Monk and R.V. Dunn, Biochimica Et Biophysica Acta-Proteins and Proteomics 1702 (2004) 103-110.
[2]V. Kurkal, R.M. Daniel, J.L. Finney, M. Tehei, R.V. Dunn and J.C. Smith, Biophysical Journal 89 (2005) 1282-1287.

Abstracts

– Multiscale simulations –

P-217

Multiscale modeling of lipids and lipid assemblies

A. P. Lyubartsev
Division of Physical Chemistry, Stockholm University, 10691, Stockholm, Sweden

A multiscale modeling approach is developed for simulations of lipids and lipid assemblies on mesoscale. First, atomistic molecular dynamics simulation of initially disordered system of lipid molecules in water is carried out. On the next stage, radial distribution functions (RDFs), obtained from the molecular dynamics simulation, are used to build a coarse-grained lipid model, with effective interaction potentials computed by the inverse Monte Carlo method. The computed effective potentials reproduce, for the coarse-grained model in an implicit solvent, exactly the same RDFs as those obtained in fully atomistic simulations. Due to a greatly reduced number of degrees of freedom, such coarse-grained model can be used to simulate systems consisting of many thousands of lipids, but maintaining the structure inherited from the atomistic model. This approach has been applied to build a 10-site DMPC lipid model from the all-atomic model defined by the CHARMM force field. The constructed lipid coarse grained model reproduces well properties of a plain lipid bilayer. A number of molecular dynamics simulations of the coarse-grained model have been carried out with the number of lipids ranging between 300 and 3500. When the lipids are initially organized in a bilayer fragment without boundary conditions, this fragment adopts a shape of a bicell or vesicle, depending on the number of lipids in the fragment. The process of self-assembly of lipid molecules starting from an unordered structure is also demonstrated, which results in different structures depending on the initial number and concentrations of lipids.

P-218

A computationally based large scale model of potassium flow in the cochlea

P. Mistrik[1], C. Mullaley[1], P. Saffrey[1], R. Nobili[2], F. Mammano[2], J. Ashmore[1]
[1]UCL Ear Institute, London, UK, [2]VIMM, Padova, Italy

Sensory transduction in the mammalian inner ear cochlea depends upon conversion of sound into an electrical signal by an organized array of hair cells, some of which act as sensory cells and some subsets of which act as mechanical amplifiers. Potassium flux through both classes of hair cells is essential for the transduction step. The endocochlear potential and potassium (K^+) transport is facilitated by concerted action of ion channels, co-transporters and gap junctions, mutations in any of which lead to changes in the cochlear K^+ homeostasis and hearing loss. While a K^+ circulating path from the stria vascularis to hair cells and a return via the fibrocytes of the spiral ligament is a generally accepted model, quantitative details are unclear. In the absence of an appropriate experimental data set we have approached this problem by computing a large scale model of K^+ circulation in the cochlea where K^+ flow is treated as electrical current. The macroscopic cochlear mechanics is implemented by using routines developed previously to model acoustic stimulation of basilar membrane. Time and frequency domain solutions of this problem are solved using a Modified Nodal Analysis method. The microscopic features of the model provide important cues about the physiological role of gap junction systems in K^+ homeostasis. It shows that gap junction conductance is essential for appropriate cochlear frequency tuning. The output of such a model is embedded in a software platform for understanding novel gene mutations which lead to changes in the homeostatic function and hearing loss.
Supported by EUROHEAR (LSHG-CT-2004-512063)

P-219

The MARTINI force field: extension to proteins

L. Monticelli[1], S.-J. Marrink[2], D. P. Tieleman[1]
[1]Dept. Biological Sciences, University of Calgary, Canada, [2]Dept. Biophysical Chemistry, University of Groningen, The Netherlands

Many biologically interesting phenomena occur on a time scale that is too long to be studied by atomistic simulations. These phenomena include the dynamics of large proteins and self-assembly of biological materials. Coarse-grained (CG) molecular modelling allows computer simulations to be run on length and time scales that are 2-3 orders-of-magnitude larger compared to atomistic simulations, providing a bridge between the atomistic and the mesoscopic scale. We developed a new CG model for proteins as an extension to the MARTINI force field for lipids. The model is computationally efficient and reproduces peptide-lipid interactions and the partitioning of amino acids and peptides in lipid bilayers. In order to validate the model, we calculated the potential of mean force for each amino acid as a function of its distance from the center of a DOPC lipid bilayer. Comparison with atomistic results shows good agreement. We used molecular dynamics simulations to study the aggregation of the WALP23 peptide in lipid bilayers. WALP23 is a helical transmembrane peptide, and in the simulations unfolding is avoided using restraints based on the peptide conformation. Experimental data shows that WALP23 forms mainly monomers and dimers in lipid bilayers. We performed microsecond timescale simulations starting from 64 monomeric helices embedded in a DOPC bilayer. Partitioning and orientation are in good agreement with previous atomistic simulations, and the aggregation behavior is compatible with available experimental data. Reversible aggregation-disaggregation of the peptides is observed on the microsecond time scale.

O-220

Ab initio studies of amyloid-metal complexes: a tool towards understanding misfolding and aggregation

S. Morante
Department of Physics University of Rome "Tor Vergata", Italy

Well identified peptides and proteins (like the Aβ-peptide and the prion protein) are known to undergo a misfolding process during the development of the associated neurodegenerative disease. An important, but not yet fully elucidated, role is played by transition metals (mainly Cu^{2+} and Zn^{2+}) that have been observed to be present in considerably large amount in neurological plaques. There exist in the literature conflicting statements about the role of Cu^{2+} and Zn^{2+} ions in providing protection against or acting as promoters of plaques formation [1,2].
An understanding of this and similar questions can be achieved by "first principle" simulations of atomistic models of the metal-peptide complexes of interest, based on *ab initio* molecular dynamics simulations of the Car-Parrinello type. We have studied the Cu coordination mode at the PrP binding sites which are located in the protein octarepeat region both in vacuum and with water in standard conditions. Simulations involving pairs of short octarepeat fragments have shown that the presence of stoichiometric cupper concentration favour aggregation [3]. Similar studies have been undertaken for Aβ-peptides complexed with either Cu or Zn.
1.F. Stellato, G. Menestrina, M. Dalla Serra, C. Potrich, R. Tomazzolli, W. Meyer-Klaucke, S. Morante (2006) *Eur. Biophys. J.* **35(4)**: 340.
2.T. Miura, K. Suzuki, N. Kohata, H. Takeuchi (2000) *Biochem.* **39**: 7024.
3.S.Furlan, G.La Penna, F.Guerrieri, S.Morante, G.C.Rossi (2007) *J. Biol. Inor. Chem.*, in press.

Abstracts

– Multiscale simulations –

P-221

Cell seeding of tissue engineering scaffolds: experimental, kinetic and computer simulation study

A. Neagu[1], O. Doagă[2], M. Neagu[1], T. Savopol[2], E. Kovács[2]

[1]Department of Biophysics and Medical Informatics, Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania., [2]Department of Biophysics and Cell Biotechnology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Tissue engineering aims at growing functional multicellular constructs suitable for regeneration, repair, or replacement of damaged tissues. Porous, biocompatible scaffolds support cells and allow for nutrient exchange. Cell seeding of scaffolds, the object of our study, is known to influence the properties of engineered tissues. In order to characterize the kinetics of cell attachment onto a scaffold, using an optical density meter, we monitored the concentration of the cell suspension. We verified the linear relationship between the suspension's concentration and optical density, and checked that cells did not attach to the walls of the culture flask. In order to fit the observed kinetics, we generalized Langmuir's adsorption theory to also include integrin recovery after trypsin/EDTA treatment. The kinetic equations were solved numerically, and the model parameters were determined by a nonlinear least squares method based on the Nelder-Mead simplex algorithm. The kinetic model gave a good description of the time course of cell seeding. Seeking spatial information, we simulated cell seeding by the Monte Carlo method based on a cubic lattice model of the system. We studied patterns of scaffold invasion by cells versus cell-cell and cell-scaffold interaction energies, and also investigated the impact of scaffold porosity. Since the simulations did not involve a time variable, they were complementary to the kinetic study.

P-222

Kinetics of platelet aggregation: analytic and computer simulation study

M. Neagu[1], E. Flenner[2], O. Munteanu[1], A. Neagu[1]

[1]Department of Biophysics and Medical Informatics, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania, [2]Department of Physics & Astronomy, University of Missouri, Columbia, MO, USA

Platelets are responsible for the repair of damaged blood vessels in a process that involves their adhesion to the site of injury and subsequent aggregation. A method for diagnosing platelet function defects is platelet aggregometry, based on monitoring the decrease of the extinction of a platelet rich plasma (PRP) during the progress of platelet aggregation elicited by certain agonists, such as ADP, epinephrine, collagen, arachidonic acid and ristocetin. Here we present a theoretical study of in vitro platelet aggregation by a novel kinetic model, and by Kinetic Monte Carlo (KMC) simulations. The kinetic model, adapted for describing turbidimetric experiments performed on PRP, is simple enough to allow for parameter estimation by fitting experimental data. The system of coupled, nonlinear differential equations was solved numerically, and the model predictions were compared with the results of optical density measurements. The model parameters were obtained using nonlinear least squares fitting. Based on a lattice model of platelets in suspension, we simulated time evolution of the system by the KMC algorithm, obtaining the size distribution of the aggregates as a function of adhesivities. Aggregate morphologies were compared with those resulting from Metropolis Monte Carlo simulations and with experimental findings. By analyzing data on patients with various health conditions, we aim at using model parameters as diagnostic indicators.

P-223

Computational studies on the NhaA sodium-proton antiporter of *Escherichia coli*

E. Olkhova[1], C. Hunte[1], E. Padan[2], H. Michel[1]

[1]Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Max-von-Laue Str., 3, D-60438 Frankfurt am Main, Germany, [2]Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel

The crystal structure of NhaA antiporter of *E. coli* [1] provided a basis to explore the mechanism of Na^+ and H^+ exchange and its regulation by pH [2]. Titration of individual residues and of clusters formed by these residues play an important role in activating the enzyme and in the mechanism of transport. The continuum electrostatics calculations identify four clusters of tightly interacting residues as well as long-range interactions required for activation. A number of residues with extreme pK_a values can only undergo protonation/deprotonation reactions subsequent to conformational changes [3]. However, the dynamics of the pH induced changes in the proteins remained unknown. Using molecular mechanics methods, we studied the dynamic behaviour of the hydrogen bonded network in NhaA upon shifting the pH from 4 to 8. A remarkable pH-induced conformational reorganisation was found for TMSs IVp and X [4].

[1] Hunte, C., Screpanti, E., Venturi, M., Rimon, A., Padan, E. & Michel, H. (2005) *Nature* **435**, 1197-1202

[2] Padan, E. & Schuldiner, S. (1996) *Biochim. Biophys. Acta* **1185**, 129-151

[3] Olkhova, E., Hunte, C., Screpanti, E., Padan, E. & Michel, H. (2006) *PNAS* **103(8)**, 2629-2634

[4] Olkhova, E., Padan, E. & Michel, H. (2007) *Biophys. J.* **92**, 1-8

P-224

Hydrodynamic and Brownian fluctuations in sedimenting suspensions

J. T. Padding[1], A. A. Louis[2]

[1]Compuational Biophysics, University Of Twente, The Netherlands, [2]Rudolf Peierls Centre for theoretical physics, Oxford, United Kingdom

We adapt stochastic rotation dynamics, a mesoscopic computer simulation method, to colloidal suspensions, making sure length- and time-scales are carefully separated to generate the correct coarse-grained physical properties. This allows us to study the interplay between hydrodynamic and Brownian fluctuations during steady-state sedimentation of hard sphere particles for Peclet numbers (Pe) ranging from 0.1 to 15.

Even when the hydrodynamic interactions are an order of magnitude weaker than Brownian forces, they still induce backflow effects that dominate the reduction of the average sedimentation velocity with increasing particle packing fraction. Velocity fluctuations, on the other hand, begin to show nonequilibrium hydrodynamic character for Pe > 1.

J.T. Padding and A.A. Louis, Phys. Rev. Lett. **93**, 220601 (2004)

J.T. Padding and A.A. Louis, Phys. Rev. E **74**, 031402 (2006)

Abstracts

– Multiscale simulations –

P-225

Prediction of transmembrane helix-helix assembly by molecular dynamics simulations

O. Samna Soumana, N. Garnier, M. Genest
Centre de Biophysique Moléculaire – CNRS, Orléans, France

Computational methods are useful to identify favourable structures of transmembrane (TM) helix oligomers when experimental data are not available or when they cannot help to interpret helix-helix association.
We present here a global search method using molecular dynamics (MD) simulations to predict the structures of TM domain of homo or heterodimers. The present approach is based only on sequence information without any experimental data and is first applied to glycophorin A (GpA) to validate the protocol and to the HER2-3 heterodimer receptor.
The method successfully reproduces the experimental structure of the GpA_{TM} with a root mean square deviation (RMSD) of 1.5 Å. The search protocol identifies energetically stable models of the TM domain of the HER2-3 receptor dimer with favourable helix-helix arrangement, including right-handed and left-handed coiled-coils. The predicted HER2-3 TM domain structures exhibit the GxxxG-like motif at the dimer interface which is presumed to drive receptor oligomerization. We demonstrate that native structures of TM domain can be predicted without experimental data. This search protocol could help to predict structure of the TM domain of HER heterodimer family.

O-226

Nanomechanics of biomolecules

C. Schütte
Mathematics & Computer Science, Free University Berlin

The talk will demonstrate how to extract nanomechanical properties like stiffnesses and friction parameters of biomolecules from molecular dynamics simulations. The aim is to construct optimal sets of parameters from molecular dynamics time series for all important conformations of a biomolecule under investigation. Parameter optimality will be measured in a maximal likelihood estimation sense, i.e., such parameters are optimal for which the probability that the observed time series is an output of equations of motions with these parameters is the highest possible. It will be demonstrated how to derive the equation for the optimal parameter set, how these can be implemented efficiently, and how the resulting algorithm perform for moderately sized examples (12-alanine with implicit water and some B-DNA 16mer with explicit water).

P-227

fd/M13 viruses: from single molecule flexibility to mesoscale chirality ?

F. Tombolato, A. Ferrarini
Università degli Studi di Padova, Italy

The *fd* and M13 bacteriophages are rod-like, semiflexible polyelectrolytes, with large aspect ratio. The easy way they can be genetically engineered and the ability to self- organize in ordered structures make these biomolecules attractive for nanoscale devices[1]. At high concentration, water suspensions of viruses exhibit a chiral nematic phase, wherein virions are preferentially aligned along an axis, which rotates in space forming a left-handed helix[2], with a pitch of the order of 10-100 μm. The microscopic origin of this chirality is not obvious; fluctuations in the virus shape have been suggested as the responsible.[2] To explore this possibility, we have coupled a Monte Carlo sampling of configurations of the virus, described as a worm-like polymer, to a mean-field model for its orientational distribution in the chiral nematic phase. From our analysis, the extremely strong twisting ability of single virus configurations emerges, which is however washed out by the simultaneous presence of contributions of opposite handedness. Due to the lack of knowledge of single molecule elasticity parameters, only a qualitative comparison between theoretical predictions and experimental results is possible. More information, however, could become available, thanks to recent experiments on single virus optical stretching[3].

[1] S.W. Lee et al, *Science*, **2002**, 296,892
[2] E. Grelet et al, *Phys. Rev. Lett.*, **2003**, *90*, 198302; F. Tombolato et al, *Phys. Rev. Lett.*, **2006**, 96**,** 258302
[3] A.S. Khalil et al, *PNAS*, **2007**, 104,4892

P-228

Purple membrane dynamics: isotope labelling, neutron scattering and molecular dynamics simulations

K. Wood[1], B. Kessler[2], M. Weik[1], S. Grudinin[4], G. Kneller[3], M. Johnson[3], D. Oesterhelt[2], G. Zaccai[3]
[1]Institute of Structural Biology, Grenoble, France, [2]Max Planck Institute Biochemistry, Martinsried, Germany, [3]Institut Laue Langevin, Grenoble, [4]Institute for Structural Biology, Juelich, Germany

The interactions that maintain protein structures have characteristic energies in the thermal regime and on the ps-ns timescale. Molecular dynamics (MD) simulations and neutron scattering both access the ps-ns timescale, and are particularly effective complementary techniques, since calculating a simulated neutron spectrum from a MD trajectory is relatively straight-forward. Using H/^{2}H labelling allows a neutron study to focus specifically on the dynamics of different parts of a system.
We have investigated the dynamical heterogeneity of the purple membrane (PM), an organised structure of bacteriorhodopsin (BR), lipids and hydration water, using a unique combination of H/^{2}H labelling, elastic incoherent neutron scattering and MD simulations.
Neutron scattering data revealed differences in the dynamics of isoleucine, leucine and lysine residues on the ps-ns timescale as a function of temperature. MD simulations of three BR molecules inserted in a lipid bilayer and solvated, at several temperatures, show that the dynamics of isoleucine and leucine residues are well represented by usual force fields, and allow site-specific properties to be examined. The dynamics of lysine residues in the simulation and in the experiment do not compare well together however, perhaps because of their more complex interactions with hydration water.

Abstracts

– Glycobiophysics –

P-229

Utilizing computational chemistry for protein engineering – study of lectin-sugar interactions

J. Adam[1], M. Pokorna[1], C. Sabin[2], Z. Kriz[1], A. Imberty[2], J. Koca[1], M. Wimmerova[1]
[1]National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic, [2]CERMAV-CNRS, Grenoble, France

The protein engineering approach is a very valued tool, a process of designing proteins with precisely defined and desired properties. However, this approach can be quite demanding for both time and budget.
The current computational capacity allows to perform sophisticated simulations and modelling of the biomolecular interactions. The combination of practical methods, structural and functional experimental methods is crucial for understanding the relationship between structure and properties. The more complete this understanding, the easier and more effective the process of protein engineering. Lectins are proteins capable of binding saccharides with both high affinity and specificity. Saccharides, thanks to their wide variability of conformational alternatives, are able to serve as potent recognition agents.
The study of affinity changes related to mutations of lectin PA-IIL by combination of the aforementioned methods revealed the crucial role of the amino-acid composition of the binding site for binding preferences. The studied mutants were created *in silico* as well, and the resulting structures were used for docking, and the results were correlated with experimental data. The aim is to develop a reliable method of precognition of future, unknown interactions, enabling effective protein engineering approach. *Supported by Grant Agency of the Czech Republic, GD204/03/H016*

P-230

Stability investigation of tamarind seed polysaccharide under stress conditions

S. Bertini[1], G. Eisele[1], C. Foletti[1], M. Sansò[2], G. Torri[1]
[1]Ronzoni Institute, Milan, Italy, [2]Farmigea, Pisa, Italy

The primary structure of Tamarind seed polysaccharide (TSP), stored in the seed of *Tamarindus indica*, consists of a (1→4)-β-D-glucan spine with (1→6)-α-branched xylose, which is partially substituted by (1→2)-β-galacto-xylose. It is characterized by high viscosity, pH tolerance and adhesivity. Such properties led to its application as stabilizer, gelling agent and binder in the food products. Other important properties of TSP were recently identified. These include non-carcinogenicity, biocompatibility and high drug holding capacity. Since TSP is an important eccipient, our study was undertaken to highlight the stability of this polysaccharide. In particular, TSP was solubilised in different conditions of pH, temperature, pressure and in presence of hydroxyl radicals to verify the stability and characterize potential degradation products. Several techniques were applied to determine the structure of TSP before and after treatments. NMR was used to investigate the chemical modifications of primary structure of TSP, and Low Resolution NMR has been employed to study the relaxation parameters of polysaccharide in solution. The evaluation of molecular weight, intrinsic viscosity and hydrodynamic radius has been done by high performance size exclusion chromatography. Moreover, the hydrodynamic radius of TSP in solution was obtained directly from Dynamic Light Scattering measurements. We demonstrated the stability of TSP in pressure and the degradation to low molecular weight fractions, oligosaccharide and monosaccharide only in very extreme conditions of pH and temperature as in presence of radicals.

O-231

Anomalous phase behaviour of glycolipid self-assembly tuned by sugar headgroup surface arrangement.

E. del Favero[1], P. Brocca[1], L. Cantù[1], M. Corti[1], A. Raudino[2]
[1]Dept. Med.Biotechnologies, University of milano, Italy., [2]Dept. Chemical Sciences, University of Catania, Italy.

We present anomalous mesophase behavior of single component, non-added salt solutions of a family of membrane glycolipid bearing large headgroups: the gangliosides. Contrary to one of the mainstays of the colloid science, that the aggregation number of amphiphile aggregates grows with concentration, an anomalous region is found at intermediate concentrations of several of those glycolipid solutions, where a sharp decrease of the aggregation number occurs. The conventional picture of interacting micelles says that a reduction in the molecular surface area (increase of aggregate size) is paid to reduce intermicellar interactions when the solution is concentrated. Furthermore we present data on a bilayer forming glycolipid able to spontaneously vesiculate at high dilution and to concentrate in a more curved aggregate shapes (ribbons) opposite to usual paradigm telling that curvature can only decrease as concentration increases. The common point at the base of this rich phenomenology is that the amphiphilic surfaces made of bulky headgroups of more or less complex sugar chains, is able of modifying molecular surface area and protrusion from the aggregate, contributing to the energy balance between inter- and intra-aggregate interaction. This capability in the case of GM3 single component solutions have to be played non-homogeneously on the aggregate surface to account for edge energy cost of ribbons and finite membrane, and to allow for asymmetric curvature of the two monolayeres of the vesicles. We show that the different arrangements can be fine-tuned by physical condition as, very interestingly, counter-ion substitution.

O-232

Macromolecular assemblies at the cell surface: complex cell regulation directed by glycan complexity

L. Duchesne, E. A. Yates, T. R. Rudd, J. E. Turnbull, D. G. Fernig
School of Biological Sciences, Biosciences Building, University of Liverpool, Liverpool L69 7ZB, UK

Cell activity is regulated by macromolecular assemblies, which are dynamic in time, composition and location. The evolution of metazoans required the development of a sophisticated molecular machinery for mediating unprecedented levels of cell-cell communication. Key elements of this molecular machinery operate at the cell surface and in the extracellular matrix. Particular features include the glysocaminoglycans, long, linear polysaccharides, two families of which, heparan sulfates (HS) and chondroitin sulfates (CS) exhibit an incredible diversity of structures. Through their interactions with upwards of 500 proteins, HS and CS mediate the long-range assembly of the extracellular matrix (along with hyaluronic acid) and regulate the activity of the majority of protein effectors (morphogens, growth factors, cytokines, chemokines) that control cell function and fate. At the molecular level two key features of these systems stand out. Firstly, the complexity and diversity of the structures of chains of HS and CS, with new tools clearly showing exquisite biological specificity in terms of the expression of particular structures. Secondly, an apparent loss of specificity upon in vitro analysis, the consequence of using techniques which average structure and function over a population of molecules. A combination of new spectroscopic approaches with gold nanoparticle probes imaged by photothermal microscopy provides a route to understanding structure-function relationships in heparan sulfate at a level of sophistication commensurate with the complexities of its molecular functions at the cell surface and in the extracellular matrix.

Abstracts

– Glycobiophysics –

O-233

Sweet-talk between pathogen and host: structure and thermodynamics of interaction

A. Imberty
CERMAV-CNRS, Grenoble, France

Carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell in the first step of their invasion and infectivity. Although protein-carbohydrate interactions are characterized by low affinity, bacterial lectins use a variety of strategy to attain high affinity for specific binding to host carbohydrate

Lectin-carbohydrate interactions are generally characterised by a low affinity for monovalent ligands, a drawback balanced by multivalency that provides high avidity for substrates with several potential ligands available, such as complex glycans or cell surfaces. Higher affinity is obtained for longer ligand, i.e. oligosaccharides, and the interactions are typified by a favourable enthalpy term, due to the high number of hydrogen bonds, that is offset by an unfavourable entropy contribution that has been attributed either to solvent rearrangement or to loss of ligand conformational flexibility. Recent studies of bacterial lectins involved in pathogenesis and host recognition an unusually high affinity.Calcium–dependent lectins from opportunistic pathogens *Pseudomonas aeruginosa, Chromobacterium violaceaum* and *Ralstonia solanacearum* all display sub-micromolar range affinity towards their carbohydrate ligands. We used combined titration microcalorimetry and x-ray crystallography approaches to decipher the thermodynamical and structural basis for high affinity binding of bacterial lectins to host carbohydrates.

Imberty, A., Mitchell, E.P., and Wimmerová, M. (2005). *Curr. Opin. Struct. Biol.*, **15** : 525-534.

O-234

Mesoscale structure formation of polysaccharides: Induction and suppression of toroids

G. Maurstad, M. Sletmoen, B. T. Stokke
Biophysics and Med. Techn., Dept. of Physics, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

Mesoscale structure formation to build functional units of polysaccharides are essential for fulfilling their biological role (e.g. cellulose, pectins, chitin) as well as technological exploitation as drug delivery vehicles, gels, or viscosifiers. Here, the formation and suppression of toroidal structures within two classes of polysaccharide assemblies are discussed. The first is polyelectrolyte complexes formed at low concentrations. When the chain stiffness of one of the components is sufficiently large, toroids are observed as the dominating morphology by ultramicroscopic techniques. Examples include chitosan-DNA (persistence length L_p=50 nm for DNA) and chitosan-xanthan (L_p=120 nm for xanthan). The second class is the β-glucan family. Comb-like branched (1,3)-β-*D*-glucans dissolves in water as stiff triple-helical structures. Dissociation followed by re-association yield a blend of various macromolecular topologies, where the cyclic species make up a significant fraction. Re-association in the presence of certain polynucleotides yielded novel macromolecular complexes, where poly-cytidilic acid (polyC) replaces one of the glucan chains in the complex. Re-association in the presence of polyC also suppresses the abundance of the toroids compared to only β-glucan. The dimensions of the toroids in the first class is most likely determined by persistence length of stiffest component and intersegment interaction strength mediated by the electrostatic interaction. The dimensions of the toriods of β-glucans are also influenced by the chain stiffness, but lateral association beyond the triplex state is not observed in this system.

O-235

Organization of human interferon gamma -heparin complexes from solution properties and hydrodynamics

H. Perez Sanchez[2], K. Tatarenko[1], M. Nigen[1], G. Pavlov[3], A. Imberty[4], H. Lortat-Jacob[1], J. Garcia de la Torre[2], C. Ebel[1]
[1]IBS, Grenoble, France, [2]Univ. Murcia, Spain, [3]St. Petersburg Univ., Russia, [4]CERMAV, Grenoble, France

Heparan sulfate recognizes a variety of proteins including the cytokine IFNg and as such modulates many biological processes. IFNg is a homodimer with a well defined core and two flexible C-termini constituting HS binding domains. We show (1) using molecular modeling that an extended IFNg structure overlaps a HS fragment of 16 disaccharides. Since a 21-24 disaccharides HS fragment was experimentally defined as the minimum size interacting with IFNg (2), the question of the complexe organization is raised. We combine analytical ultracentrifugation, size exclusion chromatography and hydrodynamic bead modeling to characterize the complexes formed in solution with heparin oligosaccharides. For oligosaccharides of 14 and 20 nm, 2 types of complexes are formed with 1 IFNg and 1 or 2 heparin molecules. Complexes consisting of 2 IFNg and 1 or 2 heparin molecules are present for a fragment of 25 nm and aggregates for a fragment of 35nm. The complexes are rather compact and can be formed without major conformational changes of the partners. The complex pattern of interaction is related to the size of the partners and their multiple binding possibilities. These various possibilities suggest networks of interactions at the crowed surface of the cells. Hydrodynamic methods used here proved to be very efficient tools to describe protein-HS complexes that are otherwise very difficult to analyze.

(1) Perez Sanchez et al. Biochemistry, 2007, 45 13227
(2) Lortat-Jacob et al. 1995 Biochem J 310 (Pt 2) 497

P-236

Passing through Molten Globule-like State during glycation of Human Serum Albumin after 21 days incubation

N. Sattarahmady[1], A.-A. Moosavi-Movahedi[1], M. Habibi-Rezaei[2]
[1]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, [2]Department of Biology, Faculty of Science, University of Tehran, Tehran, Iran

Interaction of reducing carbohydrates with proteins leads to a cascade of reactions that are known as glycation or Maillard reaction. Here the impact of incubation of 630 mg/dl glucose with human serum albumin (HSA) at different times, on the extent of HSA glycation and structural changes was studied using circular dichroism (CD), fluorescence and microviscometer techniques. The moles of glucose bound to each mole of HSA, formation of fluorescence AGE and Amadori products during glycation were measured. Structural information besides stokes radius and ANS binding data indicates the formation of molten globule-like state at 21^{st} days of incubation of 630 mg/dl glucose with HSA. Therefore, results show probably, in acute diabetes mellitus patients, glycated HSA after 21 days of glycation pass through molten globule-like state that introduce as agent of some structural protein disease.

Abstracts
– Glycobiophysics –

P-237

Glycan dependent Notch-Notch ligand interaction

J. Shim[1], B. Petryniak[3], N. B. Holland[2], J. B. Lowe[3]
[1]Biomedical Engineering, School of Engineering, University of Michigan, Ann Arbor, Michigan, U.S.A., [2]Chemical and Biomedical Engineering, School of Engineering, Cleveland State University, Cleveland, Ohio, U.S.A., [3]Department of Pathology, School of Medicine, Case Western Reserve University, Cleveland, Ohio

Notch receptors are heterodimeric transmembrane glycoprotein receptors which are essential to cell fate determination in a wide array of developmental processes. The extracellular domain of Notch1 (ECN) contains 36 epidermal growth factor-like (EGF) repeats, many of which are heavily glycosylated with O-fucose, O-glucose, and N-glycans. ECN is modified by Fringe glycosyltransferases which elongates O-linked fucose by the addition of a beta1,3-GlcNAc on many of the EGF-like repeats. It has been reported that the glycosylation states of ECN at the O-fucose and Fringe modification sites can modulate the signal transduction events that occur when Notch interacts with Notch ligands. However, it remains unclear whether the glycosylation states of ECN modulate Notch signal transduction by altering the binding strength between Notch and Notch ligands directly, and/or some other aspects of the Notch-Notch ligand interaction (i.e. by changing the conformation of Notch).
In this work, we will use Surface Plasmon Resonance (SPR) to observe the kinetics (association and dissociation rate (ka and kd)) of the Notch-Notch ligand binding and the dissociation force by Atomic Force Microscopy (AFM) in order to understand how the glycan influences Notch-Notch ligand binding.

O-238

Characterisation of glycans and carbohydrate-binding proteins off chips: new mass spectrometry tools

B. Tissot[1], R. Karamanska[2], S. M. Haslam[1], H. R. Morris[1], D. A. Russell[3], R. A. Field[2], A. Dell[1]
[1]Imperial College London, U.K., [2]John Innes Centre, Norwich, U.K., [3]University of East Anglia, Norwich, U.K.

The multidisciplinary UK GlycoArrays Consortium is devoted to the development of carbohydrate microarray technologies and associated analytical methodologies especially mass spectrometry. Among the various goals of this consortium, the identification of new carbohydrate-binding proteins (CPB), as well as the *in situ* characterisation of glycans are two of the most challenging. Mass spectrometry is the most powerful technique for glycan and protein structural analysis and Matrix Assisted Laser Desorption Ionization (MALDI) is the method of choice for on-chip analysis, enabling new label-free strategies to be designed. We have developed MALDI-based methodologies to detect CBP immobilised on BIACORE sensorchips, previously functionalised with various types of self-assembled monolayers (SAM) and presenting covalently bound glycans. The use of MALDI TOF/TOF MS/MS instrumentation allows the unequivocal identification of minute amounts of bound proteins.
With respect to *in situ* glycan analysis, we characterised gold surfaces functionalised with different self-assembled monolayers, including sialylated SAMs. Furthermore, we applied these new methodologies to other nano-tools developed by the UK GlycoArrays Consortium such as gold glyco-nanoparticles.

P-239

Structural nanoinvariant of the giant proteoglycan macromolecule of extracellular matrix and mucins

A. Vazina[1], N. Lanina[1], A. Matyushin[1], V. Korneev[1], E. Maevskii[1], A. Vasilieva[1], A. Zabelin[2], O. Nayda[2], A. Gadzhiev[1], V. Letyagin[3], D. Burov[3], E. Polyakova[1]
[1]Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russia, [2]RRC "Kurchatov Institute", Moscow, Russia, [3]Blokhin Science Center of Oncology, Moscow, Russia

About 500 samples of intact and cancer transformed epithelial tissues and mucus from different departments of gastrointestinal tract were systematically investigated by methods of small-angle X-ray diffraction and fluorescence using synchrotron radiation. The X-ray patterns of tissues and mucins display a large number of sharp diffraction rings at spacings of 4.5 nm and its higher orders. The 4.5 nm periodicity was attributed by us to interchain spacing of polysaccharide chains covalently connected to protein core. A correlation between an integral intensity of X-ray patterns and elemental content was observed; calcium was found to be the major element of mineral composition. It was shown that polyanion proteoglycan structures can be reversibly transformed by salt solutions or chelating agents, however these structures are very stable during heating to more than a hundred degrees. The 4.5 nm reflection should be considered as a marker of solely structural modifications of intact tissue under various exogenous influences.
Comparative analysis of the X-ray patterns of different samples of mucus and biological tissues showed that the 4.5 nm spacing is a structural nanoinvariant of proteoglycans of both mucus and extracellular matrix of epithelial tissues. Schematic nanoscale periodic model of the giant proteoglycan macromolecule is considered.
The research was supported by RFBR Grant # 05-02-17708.

P-240

Lubrication of short-side-chain mucin is facilitated by its complex adsorption

G. E. Yakubov[1], J. Mc Coll[2], J. H. H. Bongaerts[1], J. Ramsden[2]
[1]Unilever Corporate Research, Colworth Park, Sharnbrook, MK44 1LQ, UK, [2]Department of Materials, Cranfield University, MK43 0AL, UK

We investigate the adsorption of the short-side-chain glycoprotein mucin (M_w = 0.55 MDa) onto hydrophobic and hydrophilic substrates (OWLS, AFM) and its impact on the tribological properties of a compliant tribological contact. Mucin adsorbs at solid-liquid interfaces in two stages as the bulk concentration is increased. Subsequent desorption depends on the bulk concentration during adsorption, which suggests non-equilibrium processes are involved. Adsorbed mucin forms a composite structure, with a tightly bound equilibrium layer adjacent to the surface and superimposed on this a structurally distinct second layer, which is not equilibrated with the underlying layer and whose internal structure is governed by entanglement. The tribological experiments demonstrate that mucin efficiently reduces boundary friction. The physically adsorbed layers of mucin form a water-swollen layer (thickness ∼14 nm) with a higher effective viscosity than the bulk viscosity. This viscous layer supports the load on the contact, prevents surface-surface interaction and thereby reduces friction. We propose that the top layer may be removed by the rubbing process, but this is then followed by a re-adsorption onto the exposed 'equilibrium' layer. This proposed non-equilibrium multi-layered structure has advantages from a biolubrication point of view, as it provides a mechanism for repair of lubricating layers involving biosurfaces and biofluids.

Abstracts

– Macromolecular assemblies –

P-241

The antimicrobial peptide PGla can adopt three distinct alignment states in lipid bilayers

S. Afonin[1], S. Grage[1], M. Ieronimo[1], P. Wadhwani[1], J. Bürck[1], P. Tremouilhac[1], E. Strandberg[1], P. Mikhailiuk[2], I. Komarov[2], A. Ulrich[1]
[1]KIT, Karlsruhe, Germany, [2]T. Shevchenko Univ., Kyiv, Ukraine

By determining the alignment of an amphipathic antimicrobial peptide in model lipid bilayers particular permeabilization mechanism ("carpet", "worm-hole", "barrel-stave") can be deduced. In oriented multibilayers with controlled temperature and hydration, even transient alignment states can be trapped and structurally characterized. This way we showed that PGLa, an antimicrobial peptide from frog, undergoes a concentration dependent re-alignment from a flat surface-bound "S-state" to an obliquely tilted "T-state" (Glaser et al, 2005). Recently, we detected a transmembrane inserted "I-state" in the presence of magainin-2, the natural synergistic partner of PGLa (Tremouilhac et al, 2006). Here, we show that PGLa is able to adopt all the three alignments (S-, T- and I-states) on its own. For the system PGLa/DMPC/H_2O we demonstrate thermotropic state conversions, which correlate with the lipid polymorphism. The peptide is always structured as an α-helix and the lipids preserve their lamellar association state. At the sub-gel lipid phase, PGLa reversibly aggregates with no preferred alignment. When the lipids are in the gel state, non-rotating homodimers of PGLa assemble into the I-state. Around and immediately above the main lipid phase transition temperature, the peptide forms tilted homodimers which are rotating about the membrane normal. When the temperature is raised further, they convert into rapidly rotating S-state monomers. We therefore conclude that S, T and I are intrinsic states of PGLa and possibly other amphipathic helical peptides in lipid membranes.

P-242

3D structure of muscle myosin filaments by electron microscopy and single particle analysis

H. A. Al-Khayat[1], E. P. Morris[2], J. M. Squire[3], R. W. Kensler[4], S. B. Marston[1]
[1]Faculty of Medicine, Imperial College London, U.K., [2]Institute of Cancer Research, U.K., [3]University of Bristol, U.K., [4]University of Puerto Rico Medical School, Puerto Rico

All muscles involve the interaction between two sets of filaments, actin and myosin, that leads to muscle contraction and force production mediated by the hydrolysis of ATP. Actin filament structure is rather well understood to high resolution by X-ray crystallography and electron microscopy (EM), but myosin filament structure is much less well defined. The myosin filaments are formed from complicated arrangements of myosin molecules and accessory proteins (e.g. C-protein and Titin). Our aim is to study and compare the arrangement of the myosin heads in the different species and how these arrangements change in diseased muscle. Mutations in cardiac muscle myosin and its associated proteins (e.g. C-protein) are known to be associated with a number of myopathies (e.g. familial hypertrophic cardiomyopathy and dilated cardiomyopathy) which change the proteins involved in producing and regulating heart muscle contraction. Having defined the structure of myosin filaments of various muscles from different species, both vertebrates and invertebrates by EM and single particle analysis. We are now studying the structure of myosin filaments in heart muscles of mammals such as mouse, rabbit and more importantly human using the well established single particle methodology. Successful detailed analysis of the structure of myosin filaments in normal heart muscle will also permit the structural effects of known myosin filaments-associated mutations to be investigated.

P-243

Structural study of Archaea viruses using electron microscopy, image analysis and 3D reconstruction

R. Aramayo[1], T. Basta[2], M. Häring[3], R. Rachel[3], D. Prangishvili[2], E. Larquet[1], N. Boisset[1]
[1]Institut de Minéralogie et de Physique des Milieux Condensés, Paris, France, [2]Institut Pasteur, Paris, France, [3]Department of Microbiology, University of Regensburg, Germany

Studies on viral diversity in extreme hydrothermal environments led to the isolation of a wide range of Archaea double-stranded DNA viruses with highly diverse morphotypes. In this work, two archaeal viruses were studied using cryo-electron microscopy, negative staining and image analysis.
The first one, AFV3 (*Acidianus Filamentous Virus*), is a novel virus infecting hyperthermophilic species of the genus *Acidianus* and is assigned to the archaeal viral family *Lipothrixviridae*. AFV3 virions were purified and analysed: they are 2000 nm long and 20.5 nm wide. Their structure consists of an outer cylindrical shell, 3.1 nm thick, containing helically arranged globular subunits and lipids. The small radius of the outer shell is inconsistent with the presence of a normal lipid layer. This shell encases an inner core containing two parallel rows of protein subunits in a zipper-like arrangement. Comparing with another known lipothrixvirus (SIFV), these subunits could be associated and wrapped with DNA.
The other virus studied is SIRV2 (*Sulfolobus Islandicus Rod-Shaped Virus*) and is a member of the family *Rudiviridae*. The size of its genome is 35.8 kbp and virions consist of a non-enveloped tube-like superhelix formed by a single protein associated with the double-stranded DNA. The helix has a pitch of about 4.5 nm. SIRV2 is 22 nm wide and 900 nm long. SDS-PAGE shows that one protein, with molecular mass of 20 KDa, is the major component of the helical tube. This protein is strongly basic and interacts with double-stranded DNA, forming a DNA-protein superhelix. The analysis of its structure is still on process.

P-244

Structure of the flavivirus NS3 protease-helicase

R. Assemberg[2], T. S. Walter[2], R. Tuma[3], J. M. Grimes[1], R. Owens[2], D. I. Stuart[1], E. J. Mancini[1]
[1]Division of Structural Biology, The Henry Wellcome Building for Genomic Medicine, Oxford University, Roosevelt Drive, Oxford, OX3 7BN, U.K., [2]Oxford Protein Production Facility, The Henry Wellcome Building for Genomic Medicine, Oxford University, Roosevelt Drive, Oxford, OX3 7BN, U.K., [3]Institute of Biotechnology and Department of Biological and Environmental Sciences, Viikki Biocenter P.O. Box 65, Univ. of Helsinki, Finland

Murray Valley Encephalitis virus (MVEV) is a member of the *Flavivirus* genus and is an Australian variant of West Nile virus. The frequency of Flavivirus infections in humans has increased in recent years and the geographical distribution is rapidly expanding to the Western hemisphere. The lack of vaccines or therapeutic treatments is prompting the elaboration of extensive prevention strategies. MVEV RNA is translated into a polyprotein that during maturation is cleaved into functional components. Non-structural protein 3 (NS3) is a multifunctional enzyme with serine protease and DEXH/D-box helicase domains, whose activity is central to flavivirus replication and therefore a possible target for the rational development of anti-flaviviral compounds. We report here the 1.85Å crystal structure of the NS3 helicase domain and the 2.85Å structure of the $NS2B_{45}$-NS3 from MVEV together with characterization of their ATPase activity. The MVEV $NS2B_{45}$-NS3 structure constitutes the first atomic view of a full-length Flavivirus NS3 protein, which coupled with the high-resolution structure of the NS3 helicase domain, suggests implications for the helicase catalytic activity, protein interactions and proteolytic processing.

Abstracts

– Macromolecular assemblies –

P-245

The structure and dynamics of intramolecular hydrogen bonds in different helical protein structures

A. Bende
National Institute for R&D of Isotopic and Molecular Technologies, Donath Street, No. 71-103, Cluj-Napoca, Romania

The properties of a protein are generally determined by its three-dimensional geometry structure. The shape into which a protein naturally folds is known as its native state. Although many proteins can fold unassisted simply through the structural propensities of their component amino acids, others require the aid of molecular chaperons to efficiently fold to their native states. One can refer to four distinct aspects of the three-dimensional protein's structure: primary, secondary, tertiary and quaternary structures. While the primary structure is given by the simple amino acid sequence, the secondary structure is defined as the local conformation of its backbone, which can form in many cases a helical structure. The aim of the present study is to give an accurate description of the molecular interactions which define the alpha-helix and 3-10 helix form polypeptide structures using the Mayer's Chemical Energy Component Analysis as well as to determine the anharmonic corrections of normal mode frequencies which is characteristic to the intramolecular -C=O...H-N- hydrogen bond vibrational motions. Accordingly, the Hartree-Fock and Density Functional Theory methods with the Mayer's CECA computer code were applied in order to obtain the atomic pair energies and the anharmonic correction technique of the harmonic vibrational frequencies implemented in the Gaussian03 computer code were used for the vibrational frequency corrections.

P-246

Electronic structure and driving forces in butylparaben:α-cyclodextrin inclusion complexes

D. S. Bogdan, C. I. Morari
National Institute for Research and Development of Isotopic and Molecular Technologies, Donath St. 71-103, 400293 Cluj-Napoca, Romania

Pharmaceutical excipients, cyclodextrins (CDs) are macrocyclic oligosaccharides composed of α-1,4-linked D-glucopyranose units. Butylparaben (BP) is an alkyl ester of 4-hydroxybenzoic acid, HO-C6H4-CO-OR (R = Bu), well known as antibacterial and antifungal agent. BP can accumulate intact in the body from the long-term, low-dose levels to which humans are exposed (cosmetics, pharmaceutical formulations, food products, beverages). A reduction in side-effects can be achieved by the increase of BP solubility in water due to the formation of inclusion complexes BP:α-CD. The geometry and electronic structure of the complexes is investigated by accurate DFT calculations. The influence of solvent upon the electronic structure of the complex is explicitly taken into account. This investigations allow us to draw meaningful conclusions upon the stability of the complexes and the nature of the driving forces that lead to the complexation process. In particular we emphasize the role of the water in the process, by pointing out the changes in the electronic structure of the solvent for different docking geometries.

P-247

Unravelling Sigma 54 transcription initiation using single particle cryo-em

D. Bose, T. Pape, M. Buck, X. Zhang
Imperial College London, U.K.

Transcription is a fundamental process of life, enabling an organism to utilise the genetic information stored in DNA to respond to a changing environment. It is essential that transcription is tightly regulated, and this is achieved in bacteria through the use of a number of dissociable sigma (σ) factors, which bind to RNA polymerase (forming a holoenzyme) and locate it to a given promoter sequence. In the majority of cases involving the predominant σ^{70} class of σ factor this is sufficient to cause transcription initiation. However, the alternative σ^{54} factor is distinct in its ability to form a transcriptionally incompetent closed complex at the promoter. σ^{54} transcription activation requires the involvement of specialised activator proteins, which are members of the AAA+ family of ATPases. These bind to DNA upstream of the promoter bound holoenzyme and contact it by looping out the intervening DNA, whereupon they use the energy from ATP hydrolysis to re-model the closed complex and thus activate transcription. Using the ATP hydrolysis transition state analogue ADP.AlF_x and activator Phage shock protein F (PspF), we have obtained 3d reconstructions of the activator bound to the closed promoter complex and of the σ^{54} holoenzyme using single particle cryo-em. These have provided insights into the mechanism of σ^{54} transcription initiation.

P-248

Effect of mechanical properties of phospholipid membranes on vesicle self-reproduction

B. Božič[1], S. Svetina[2]
[1]Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Lipičeva 2, SI-1000 Ljubljana, Slovenia, [2]Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia

Conditions for self-reproduction are sought for a growing vesicle with its growth defined by an exponential increase of vesicle membrane area and by adequate flow of the solution across the membrane. We also take into consideration the exchange of phospholipid molecules between the two membrane layers, with their net flow proportional to the difference between lateral tensions of the two membrane layers. In the first step of the presumed vesicle self-reproduction process, the initially spherical vesicle must double its volume in the doubling time of the membrane area and, through the appropriate shape transformations, attain the shape of two equal spheres connected by an infinitesimally thin neck. The second step involves separation of the two spheres and relies on conditions that cause the neck to be broken. In this paper we consider the first step of this self-reproduction process for a vesicle suspended in a solution whose solute can permeate the vesicle membrane. It is shown that the occurrence of vesicle self-reproduction depends on membrane hydraulic and solute permeabilities, the external solute concentration, the mechanical properties of the membrane, and the membrane area doubling time.

Abstracts

– Macromolecular assemblies –

P-249

Influence of p24 phosphorylations on the stability of HIV-1 capsid approached by molecular dynamics

S. Brun, E. Bernard, B. Gay, C. Devaux, C. Lionne, L. Briant, L. Chaloin
UMR 5236, CPBS, Institut de Biologie, Montpellier, France.

Phosphorylation is a key event in regulation of many cellular processes like cell cycle regulation but it also plays an important role in many virus life cycles. Here, we focused on the HIV-1 CAp24^{gag} capsid protein that constitutes the elementary unit of the capsid shape or viral core. Previous biochemical work has demonstrated the incorporation of human kinases in newly produced viruses and, more importantly, that some serine residues of the CAp24^{gag} protein were phosphorylated in vitro and in HIV infected cells. Mutations of serine 109, 149 or 178 by alanine resulted in different viral core morphologies and stabilities. Whereas the mutation S178A had no or little effect on the conical /tubular core formation as monitored by *in vitro* association, the others mutants either reduced (S109A) or completely abolished (S149A) the rate of cones formation. The secondary structure of mutants was controlled by circular dichroïsm in order to ensure that mutations did not induce any unfolding. The role of these functional residues, and particularly their phosphorylation state, was addressed by molecular dynamics simulations (quasi-harmonic and normal modes approaches). Simulations over 5 ns in water suggested that S149 plays a role in the stabilisation of the hexameric ring. Because protein motions are essential for the organisation of the capsid (assembly and maturation), the collective motions of p24 hexamers have been calculated by including one of the three phosphorylated serines. Preliminary results showed that the amplitudes of the rigid body motions are affected especially for the carboxy-terminal domain, but only for certain phosphorylations.

O-250

An automated pipeline for macromolecular microscopy: case studies

B. Carragher, A. Cheng, D. Fellmann, C. Irving, G. Lander, S. Nayak, J. Pulokas, J. Quispe, S. Stagg, N. Vossman, C. Yoshioka, C. Potter
National Resource for Automated Molecular Microscopy, The Scripps Research Institute, La Jolla, California, USA

Over the past decade, cryo-electron microscopy (cryoEM) has emerged as a powerful approach to the structural determination of large macromolecular complexes. Elucidating the structure and mechanism of action of these "molecular machines" is an emerging frontier in understanding how the information in the genome is transformed into cellular activities. In cryoEM the macro molecular specimen is preserved in a thin layer of vitreous (glassy) ice and imaged in the electron microscope using very low doses of electrons. The low signal to noise ratio of the resulting images means that averaging is required to recover the signal and reconstruct a three dimensional map of the structure.
Our goal is to develop a pipeline to automate the processes involved in solving macromolecular structures using cryo-electron microscopy. One of the goals of the pipeline is to enable much higher data throughputs and improve the resolution of single particle reconstructions. We are also using the pipeline to help understand what currently limits resolution in these maps. The current status of these efforts will be illustrated using a variety of macromolecules as case studies.

P-251

Linear dichroism: recent developments in the study of the of membrane and fibrous proteins

T. R. Dafforn[1], M. R. Hicks[3], R. Mortimer[2], E. Mortimer[2], R. Marrington[3], S. G. Addinall[4], A. Rodger[3]
[1]Biosciences, U. of Birmingham, UK, [2]Dioptica Scientific, Rugby, UK, [3]Department of Chemistry, U. of Warwick, UK, [4]Faculty of Life Sciences, U. of Manchester, UK

Understanding the organization of molecules in naturally occurring ordered arrays (e.g. membranes and protein fibres) is of great importance to understanding biological function. Unfortunately, few biophysical techniques provide detailed structural information on these non-crystalline systems. UV linear dichroism (LD) provides such information but has been severely under utilised in the past. We have made significant advances in instrumentation and methodologies that, at last, allow this potential to be fulfilled.
INSTRUMENTATION: We have developed a new type of LD Couette that requires much reduced sample concentration and volume while retaining the sample alignment required for LD measurements.
METHODOLOGIES We have demonstrated that LD can now provide a detailed understanding of the molecular mechanisms of such fundamental biological processes as protein fibre formation and membrane protein function and folding.
Protein fibres. We have shown that LD can provide the orientation of secondary structure elements and aromatic side chains in amyloid and globular fibres.
Membrane proteins. LD can provide information on the orientation of secondary structural elements in proteins bound to membranes. We have used this to study the structure of membrane proteins and the mechanism of their insertion into the membrane.

P-252

Spatial Control of Bacterial Cytokinesis: MinC antagonizes the scaffolding activity of FtsZ

A. Dajkovic[1], L. Ganhui[2], S. Sun[2], D. Wirtz[2], J. Lutkenhaus[3]
[1]Institut Curie, Paris, France, [2]Johns Hopkins University, Baltimore, Maryland, U.S.A., [3]University of Kansas Medical Center, Kansas City, Kansas, U.S.A.

Spatial control of cytokinesis is essential for many fundamental biological processes. In many bacteria, the Min system contributes to proper positioning of the cytokinetic Z ring by preventing its assembly on the poles of the cell, but the mechanism is unknown. Here we report that the effector of the Min system, MinC, controls the scaffolding activity of FtsZ by antagonizing the mechanical integrity of FtsZ structures. MinC is a modular protein whose two domains (MinC$_C$ and MinC$_N$) synergize to inhibit FtsZ function. MinC$_C$ interacts directly with FtsZ polymers and this interaction accounts for the efficient targeting of MinC to Z rings. MinC$_C$ also has debundling activity – unique among cytoskeletal proteins – since it antagonizes lateral interactions between FtsZ polymers. MinC$_N$ contains a positively charged surface that causes a decrease in the persistence length of FtsZ polymers and the consequent shortening of polymers. These activities of MinC lead to a loss of elasticity of FtsZ polymer networks. Based on our experimental results, we implement a stochastic model of the Z ring.

Abstracts

– Macromolecular assemblies –

P-253

The crystal structure of the d(TAGG) fragment complexed with a 2,7-diamidoanthraquinone

D. de Luchi, G. Wright, J. A. Subirana
Department of Chemical Engineering Universitat Politecnica de Catalunya, Barcelona, Spain

The G-rich strand present at the end of the telomeres can fold into a four-stranded quadruplex structure. The formation of a quadruplex structure at the 3' end of telomeric DNA prevents telomerase from adding further repeats. A number of small-molecule ligands have been described that stabilise quadruplex formation, and which result in telomerase inhibition, between them there are the antraquinones derivatives.
Here we report the detailed molecular structure of the TAGG fragment of the mammalian telomere complexed with a 2,7-diamidoanthraquinone. Surprisingly, the DNA does form neither a G-quadruplex, nor a (TA)A triad as described by Kettani et al [1] for the same sequence in solution. We can say it is an inverse drug-DNA complex. The structure has been determined by single crystal X-ray diffraction. The crystal belongs to space group $P4_32_12$ with a=b=29.69 and c=60.88. Surprisingly, we found that the drug molecules, thanks to the stacking interactions between the anthraquinone core, generate a lattice that the short fragments of DNA help to maintain. The DNA stabilizes the structure thanks to its extreme flexibility and its versatile capability in forming H-bonds. In this structure we can find a wide range of the possible H-bonds that can occur betweeen the DNA bases (Watson-Crick, reverse Watson-Crick, guanine-guanine, etc).
[1] Kettani et al, Nature Structural Biology 4, 382-389, 1997.

P-254

Biophysical characterization of the notch transcriptional activation complex

C. del Bianco, S. C. Blacklow
Brigham and Women´s Hospital and Harvard Medical School, Boston, U.S.A.

Notch receptors play a central role in many cellular processes through a novel signaling mechanism that ultimately influences the differentiation and proliferation of a wide variety of cell types. Signal transduction is initiated by the binding of specific ligands to the extracellular domain of Notch, which triggers a series of proteolytic cleavages that results in the release of the intracellular portion of the receptor from the membrane. Free intracellular Notch then translocates to the nucleus and activates the transcription of target genes by forming a complex with a conserved transcription factor (CSL) and a transcriptional coactivator of the mastermind-like family (MAML). Using the crystal structure of the Human Notch1 complex as a guide, we have identified a number of residues within Notch and its partner proteins that are important for complex formation. These positions were probed by site-directed mutagenesis and evaluated by a variety of *in vitro* biophysical techniques and cell based assays. We have also begun to investigate how CSL and Notch cooperate in the recruitment of MAML. These studies combine structural analysis with quantitative biochemical methods, such as FRET and EMSA, to measure the binding affinities of each component protein for their respective partner proteins. The data will help to build a model for the step-wise assembly of the ternary complex.

P-255

Fleximags to study the mechanics of bio-molecular assembly

O. Du Roure[1], J. Heuvingh[1], A. Rousselet[2], M. Fermigier[1]
[1]PMMH, ESPCI, Paris, France, [2]Institut Curie, Paris, France

Fleximags are one-dimensional structures formed of magnetic colloids linked by the studied objects. Their dynamic behavior results from the coupling between their flexibility (due to the elasticity of the linkers), their magnetic properties and the viscosity of the fluid. Its study gives access to the mechanical properties of the linker with various advantages: first, many objects are put in series resulting in the amplification of a nanometric deformation in a quantity easily measurable by optical microscopy; second the fleximag is the measuring instrument itself, which enable multiple parallel measurements and hence a good statistics.
Live cells are constantly exposed to mechanical forces produced by flow, adjacent cells or their generated own contractility. Understanding how cells are able to measure forces and how they regulate intracellular processes is of prime interest. First step is the measurement of the mechanical properties of biomolecular assemblies and their regulation by the medium. Our approach focuses on two systems: Centrosomes and actin gels. The centrosome anchors and nucleates microtubules and is always located in the center of a moving cell. It shows a huge plasticity during cell cycle suggesting an elastic behavior. Its mechanics has not yet been studied because of its complex structure. Actin gels are the main constituent of cell's cortex. We have recently created fleximags with highly branched actin gels as the linker. We are investigating the mechanical properties of the actin network and its variations due to crosslinking and degree of branching, as well as the reorganisation dynamics of the network in response to the applied deformations.

P-256

Thermodynamic protein-protein interaction studies on Pdx1 and Pdx2 from *Plasmodium falciparum*

K. Flicker[1], M. Neuwirth[1], I. Tews[2], B. Kappes[3], P. Macheroux[1]
[1]Technische Universität Graz, Institut für Biochemie, A-8010 Graz, Austria, [2]Universität Heidelberg, Institut für Strukturbiologie, Biochemiezentrum der Universität Heidelberg, D-69120 Heidelberg, Germany, [3]Universitätsklinik Heidelberg, Abteilung für Parasitologie, D-69120 Heidelberg, Germany

Pyridoxal-5'-phosphate (PLP) is an extremely versatile cofactor and is needed for the functionality of many different enzymes. Pdx1 and Pdx2 from *P. falciparum* constitute an alternative *de novo* PLP biosynthesis pathway different from the pathway present in *E. coli.* Both proteins form a hetero-multimeric PLP-synthase complex consisting of 24 subunits with 1:1 stoichiometry. Formation of the complex was studied in the presence and absence of the glutaminase substrate L-glutamine using isothermal titration calorimetry. Presence of 1 mM L-glutamine leads to a 30-fold decrease of K_d, which indicates complex stabilisation by the substrate. Structure-based calculation of the heat capacity change (ΔC_p) using a 3D structural model of the PLP-synthase complex correlates complex structure to experimental ΔC_p and describes a protein interface which is dominated by hydrophobic interactions. Concluding from a more negative experimental ΔC_p for the interaction of *Pf*Pdx1 and *Pf*Pdx2 in the presence of L-glutamine it is evident that L-glutamine alters structural properties and/or the extent of interaction at the protein-protein interface of the protein complex, thus, leading to even more hydrophobic interaction. The role of the N-terminal αN helix of *Pf*Pdx1 in complex formation with *Pf*Pdx2 was investigated in more detail. The results indicate that αN is essential for contact formation and is important in glutaminase activation of *Pf*Pdx2.

Abstracts

– Macromolecular assemblies –

P-257

Using interdisciplinary approaches to elucidate the structure and function of the AAA ATPase p97/VCP

P. S. Freemont, L. Briggs, C. McKeown, H. Niwa, H. Yeung, P. Kloppsteck, R. Issacson, S. Matthews, X. Zhang
Centre for Structural Biology, Division of Molecular Biosciences, Imperial College, London, U.K.

p97 (VCP, Cdc48 in yeast) is an abundant protein (1% cytosol) that belongs to the AAA+ ATPase family and is involved in a number of major cellular pathways in eukaryotes. With the adaptor protein p47, the p97-p47 mediates membrane fusion events in organelle biogenesis after mitosis that includes the ER, Golgi and nuclear envelope. The other p97 adaptor complex Ufd1-Npl4 directs p97 in mediating the translocation of proteins from the ER as part of the ER Associated Degradation pathway (ERAD). The same p97 adaptor complex is also involved in spindle disassembly post mitosis. Our studies are aimed at understanding the mechanism and specificity of p97 and its adaptor complexes and involve using a combined structural biology approach comprising X-ray crystallography, cryo-EM and NMR as well as biophysical tools to understand p97 as an enzyme. Our recent results will be presented at the meeting.

P-258

The elasticity of an individual collagen fiber

S. G. Gevorkian[1], D. S. Gevorgyan[2], A. L. Simonian[3]
[1]Yerevan Physics Institute, Yerevan, Armenia, [2]Yerevan State Medical University, Yerevan, Armenia, [3]Auburn University, Auburn, U.S.A.

To measure the Young's dynamic modulus E and the logarithmic damping decrement θ of an individual collagen fiber Morozov's micromethod was applied (V.N. Morozov *et al*, Biopolymers **20**, 451, (1981)). The method is based on the analysis of electrically excited transverse resonance vibrations of plates supported as cantilevers. The method's modifications, which enables us to measure E and θ within a wide temperature range is described elsewhere (V.N. Morozov, S.G. Gevorkian, Biopolymers **24**,1785, (1985)). As a characteristic of internal friction we use logarithmic decrement $\theta = \ln(A(t)/A(t+T))$, $A(t)$ - oscillation amplitude at time t, $A(t+T)$ - the same after one period. Young's modulus for sample main axis can be calculated by formula $E = 3.19 f_0^2 l^4 \rho P / I_{min}$, l - sample length, P - area of cross-section, ρ - density, I_{min} - main inertia moment of section. The method for measuring the hydration of the samples weighting up to 0.01mg is described in (S.G. Gevorkian *et al*, Biofizika **28**, 944, (1983)). It was shown that the dependence of collagen micro-fibril mechanical properties on frequency has a complex no monotonic character, which allows choosing the required frequency range in mapping alterations in stiffness and permeability observed in glycated tissues. Bounding collagen fibrils with ferment decreases the Young's modulus. It was shown that mechanical properties of the collagen-ferment system are changing during the interaction with substrate. These changes are specific and depend on substrate concentration.

P-259

Effect of cytoplasmic tail elongation of hemagglutinin on hemagglutinin-mediated membrane fusion

M. Gotoh, T. Okada, Y. Ogawa
Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

Hemagglutinin (HA) is the major transmembrane protein of influenza virus. HA is a multifunctional protein responsible for the attachment of virons to the cell surface and for membrane fusion, which allows penetration of the viral genome into the cytoplasm. The cytoplasmic tail of HA consists of 10 to 11 amino acids, and its length has well conserved among each subtype.
To investigate the effect of cytoplasmic tail elongation of influenza virus strain A/Hong Kong/1/68 (H3N2) HA on membrane fusion activity, we constructed the expression vector for the cytoplasmic tail elongated HA with green fluorescent protein (HA-GFP). The HA-GFP was expressed in HeLa cells and its trypsin sensitivity and binding activity to chicken red blood cells were examined. Then the membrane fusion activities of HA-GFP were investigated along the sequence of HA-mediated membrane fusion events: 1) lipid-mixing, and 2) content-mixing were examined using transfer assays of lipid and aqueous dyes under a confocal laser scanning microscope, and 3) pore enlargement was examined using syncytium formation assay under a light microscope. It was revealed that the HA-GFP was cleaved into two subunits by trypsin and bound to target membrane as well as HA wild-type. However, the membrane fusion activity of HA-GFP was suppressed at the stage of lipid-mixing. Therefore, we suggested that the HA cytoplasmic tail elongation with GFP had inhibitory effect on the lipid-mixing, and a certain length of HA cytoplasmic tail is critical for the lipid-mixing step.

P-260

Interaction of PGLa with other antimicrobial peptides: How specific is synergism?

S. Grage, S. Afonin, M. Ieronimo, M. Berditsch, P. Milhailiuk, P. Wadhwani, P. Tremouilhac, E. Strandberg, A. Ulrich
Forschungszentrum Karlsruhe, Institute of Biological Interfaces and University of Karlsruhe, Institute of Organic Chemistry

Recently, synergism has been found for a number of pairs of antimicrobial peptides, such as PGLa and magainin-2. Thus not only peptide-lipid interactions, but also the interplay with further peptides or membrane proteins could be important for antimicrobial activity. To evaluate this possibility, we analyzed the behaviour of PGLa in the presence of other peptides (gramicidin-S, gramicidin-A and magainin-2 and MAP). This way we addressed both unspecific interactions, such as lateral crowding in a protein-rich environment, and specific interactions underlying e.g. synergistic activity enhancement. Using solid state 19F-NMR on 19F-labelled PGLa, three distinct states of alignment in the membrane were identified previously as a function of peptide:lipid ratio and temperature: the surface-bound S-state, the tilted T-state, and the membrane inserted I-state. In this study we used the signal of a single 19F-label as a fingerprint to monitor changes in the alignment of PGLa due to interaction with other peptides. In the presence of gramicidin-S, gramicidin-A and MAP, the PGLa alignment differed only marginally from the behaviour of PGLa alone. However, magainin-2 lead to the occurrence of the inserted I-state over a the entire PGLa:lipid and temperature range studied. This stabilization of the I-state was only observed with magainin-2, the peptide found to interact synergistically with PGLa. We thus found no significant unspecific lateral crowding effects, but a pronounced peptide-peptide interaction, specific for the PGLa/magainin-2 pair.

Abstracts

– Macromolecular assemblies –

P-261

Structural analysis of minimalist LK peptides by means of circular dichroism and Raman spectroscopy

G. Guiffo-Soh[1], B. Hernández[1], Y. Coïc[2], F. Boukhalfa-Heniche[1], M. Ghomi[1]
[1]UMR CNRS 7033, BioMoCeTi, UFR SMBH, Université Paris 13, 74 rue Marcel Cachin, 93017 Bobigny cedex, France, [2]Unité de Chimie Organique, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris cedex 15, France

Minimalist peptides formed only by leucine (L) and lysine (K) have recently shown their capability to translocate antisense DNA into glioma cells.[1] This effect was related to the capacity of LK peptides to destabilize the cell membranes.[2] To understand the structure-activity relationship of some LK peptides, we have undertaken a systematic study on the structural properties of the LK peptides with primary sequences: N^{ter}KLL(KLLL)$_n$KLLK (family 1) and N^{ter}-(KL)$_n$K (family 2). Circular dichroism (CD) and Raman scattering were used to analyze these peptides in aqueous media, in order to eliminate the eventual ambiguity concerning conformational assignments based on the use of CD spectra alone. In both families a minimal length of 15 amino acids is necessary to obtain a well defined homogeneous canonical structure, i.e. α-helix (family 1) and β conformers (family 2). Raman spectra recorded upon H-D exchange on a 15-mer and a 19-mer belonging to family 1, could provide evidences on α-helix bundle formation.

1. Boukhalfa-Heniche et al. *Biopolymers* **2004**, 73, 727.
2. Béven, L. et al. *Eur. J. Biochem*. **2003**, 270, 2207.

O-262

Following the signal sequence by cryo-EM

M. Halic[1], M. Blau[2], T. Becker[1], T. Mielke[2], M. R. Pool[3], K. Wild[4], I. Sinning[4], R. Beckmann[1]
[1]Gene Center, Univ. of Munich, Germany, [2]Inst. of Biochemistry, Charite and UltraStructureNetwork, Berlin, Germany, [3]Univ. of Manchester, UK, [4]Heidelberg Univ. Biochemistry Center, Germany

Co-translational insertion into or translocation across the membrane of membrane and secretory proteins is an essential process in all cells. This process is dependent on signal sequence recognition directly on the ribosome by the signal recognition particle (SRP) which in prokaryotes has to compete for nascent chain interaction with trigger factor (TF), a ribosome-associated chaperone. While TF interaction supports folding by avoiding co-translational aggregation of the nascent chain, stable binding of SRP to the ribosome results in targeting of the ribosome-nascent chain complex to the protein-conducting channel at the membrane.
Here, we present an ensemble of cryo-electron microscopy (cryo-EM) structures of ribosomal complexes at sub-nanometer resolution. The structure of a programmed E. coli 70S ribosome-nascent chain complex allowed the visualizing of the signal sequence at the ribosomal tunnel exit. It is likely to adopt an alpha-helical secondary structure and is observed in a distinct position at the exit. The bacterial ribosome-TF complex revealed that TF binds to the ribosome as a flexible molecule and that it removes the signal sequence from its primary position at the ribosomal exit. Structures of ribosome-SRP complexes allowed building of molecular models including a first model for the ribosome- and SRP-bound signal sequence in the context of the RNC-SRP targeting complex. Conformational changes between different functional states of components of the ribosomal exit site as well as of SRP and TF were observed and provide the structural basis for the function of these complexes in the highly coordinated interplay of protein synthesis, sorting and folding.

P-263

Time-resolved protein dynamics measured in real-time by quasielastic neutron scattering

T. Hauß[1], J. Pieper[2], A. Buchsteiner[3], R. E. Lechner[1], N. A. Dencher[1]
[1]Clemens-Schöpf-Institute, University of Technology Darmstadt, Darmstadt, Germany, [2]Max-Vollmer-Laboratory, University of Technology Berlin, Berlin, Germany, [3]Hahn-Meitner-Institute Berlin, Berlin, Germany

Upon light absorption Bacteriorhodopsin (BR) undergoes a catalytic cycle (photocycle), whereby it translocates a proton from the cytoplasm to the extracellular medium. Structural studies using neutron and X-ray diffraction revealed conformational changes during the photocycle with the most pronounced structural change in the M-intermediate and these structural changes are associated to the vectorial proton transport. It is assumed that the structural changes are accompanied by changes in the protein dynamics on a timescale of femto- to pico-seconds, and that these dynamical changes are actually necessary to facilitate the structural flexibility on longer time scales. For the study of the kinetically modulated protein dynamics, we developed, for the first time, a real-time setup for the investigation of the time-resolved protein dynamics using quasielastic neutron scattering (QENS). For this experiment laser pulses, which initiate the photocycle, were synchronized with the neutron pulses of the time-of-flight spectrometer NEAT. The delay between the laser pulse and the neutron pulse was optimally chosen to probe the dynamics in the M intermediate according to results of time resolved absorption spectroscopy on the same BR sample. With this technique we are able to see, from a dynamical point of view, a protein at work. The implications on the structure-dynamics-function relation are discussed.

P-264

Elastic behaviour of fibrin fibres by means of high frequency single particle tracking

M. Jahnel, T. A. Waigh, J. R. Lu
Biological Physics, School of Physics and Astronomy, University of Manchester, Manchester, UK

Fibrin fibres are the core component of mammalian blood clots and their elastic properties are essential for the performance of blood clotting mechanisms. We study the dynamics of individual fibres *in vitro* by means of a single particle tracking experiment. The movement of optically resolved carboxylated microspheres attached to the polymer network is observed with light microscopy using a high-speed Photron CCD camera (0.01 – 10^4 Hz). The thermally driven fluctuations of the microspheres are analysed to obtain the mean-square displacement (MSD), from which the elastic properties of individual fibres and the microrheological properties of the network may be extracted (1, 2). The study also provides a test for the theoretical predictions of scaling behaviour of branched semi-flexible polymers at short timescales (MSD $\sim t^{3/4}$), and allows for the investigation of inherent errors in particle tracking routines (3).

References

(1) A. Caspi et al., Semiflexible Polymer Network: A View From Inside, Phys. Rev. Lett. 80, 1106 - 1109 (1998)
(2)T. A. Waigh, Microrheology of complex fluids, Rep. Prog. Phys. 68, 685 – 742 (2005)
(3)T. Savien, P. S. Doyle, Static and dynamic errors in particle tracking microrheology, Biophys. J. 88, 623 – 638 (2005)

Abstracts

– Macromolecular assemblies –

P-265

Characterization of a geminivirus coat protein expressed in Schizosaccharomyces pombe

K. Kittelmann, H. Jeske
University of Stuttgart, Institute of Biology, Department of Molecular Biology and Plant Virology, Stuttgart, Germany

The coat protein of geminiviruses exhibits important functions for encapsidation of the viral genome, transmission by the insect vector and transport of the viral DNA into and out of the nucleus. To analyze these functions and to establish an in vitro assembly system for high resolution electron cryo-microscopy, the coat protein of African cassava mosaic virus (ACMV) was expressed in the yeast Schizosaccharomyces pombe. Cesium sulfate gradient centrifugation revealed free protein for the lysate supernatant of the coat protein expressing cells whereas protein in the pellet fraction was partially bound to nucleic acids. In contrast, coat protein in the supernatant fraction eluted in the void volume when applied to a small Superdex 200 gel filtration column indicating higher aggregates or binding to nucleic acids. To discriminate between coat protein pentamers and coat protein bound to nucleic acids the cell lysate supernatant was separated on Superose 6 HR 10/30 in the presence of 0.6 M NaCl. Here again, the coat protein was not present as monomers or dimers but eluted in the void volume as well as in volumes corresponding to approximately 400 kDa. In addition, attempts were made to solubilize the coat protein of the cell lysate pellet. The coat protein expressed in S. pombe will be further analyzed for its ability to bind to ssDNA and to assemble into particles.

P-266

NMR analysis of β subunit conformation in subcomplexes of F1-ATP synthase

M. Kobayashi[1], H. Yagi[1], T. Yamazaki[2], M. Yoshida[3], H. Akutsu[1]
[1]Institute for Protein Research, Osaka Univ., Japan, [2]RIKEN G.S.C., Japan, [3]Natural Resources Laboratory, T.I.Tech., Japan

H^+-ATP synthase is a large multisubunit enzyme that consists of membrane-embedded F_o and F_1 ($\alpha_3\beta_3\gamma\delta\varepsilon$), which synthesizes/hydrolyzes ATP coupled with H^+-translocation. The crystal structure of MF_1 has reported, which showed that three α and β subunits were located alternatively in a ring formation penetrated its central hole by γ subunit. The catalytic site is at the interface of α and β subunits. In 1997, the rotation of $\alpha_3\beta_3\gamma$ was proved to be coupled with ATP hydrolysis. Through the reaction, F_1 takes ATP into its catalytic site, hydrolyzes and releases ADP and Pi coupling, with the rotation of γ subunit as a shaft of the F_1 motor. The conformation of β subunit changes during the reaction, from the open to closed forms on nucleotide binding. However, this successive reaction has never been studied as a whole. For this purpose, clarifying the F_1solution structure at work is necessary. We're trying to approach it by investigation of F_1 subcomplexes by NMR. However, the measurements of large molecules in solution are very difficult. To improve the quality of spectra, CRINEPT and segmental-labeling by intein was brought in. The molecular mass of β subunit is 52 kDa, while the final goal is the smallest unit of rotational catalytic system, $\alpha_3\beta_3\gamma$ complex (350kDa). To tackle this target, we have started from working on $\alpha_3\beta_3$ hexamer. Since the $\alpha_3\beta_3$ has close molecular mass to that of $\alpha_3\beta_3\gamma$ and 3-fold symmetry, it is suitable for exploring analytical method. Although it turned out that $\alpha_3\beta_3$ are in the equilibrium with α and β monomers and hetero dimers, each of their signals could be identified and assigned.

P-267

The structural adaptation of DNA in columnar assemblies and its consequence for X-ray diffraction.

A. A. Kornyshev[1], D. J. Lee[1], S. Leikin[2], A. Wynveen[1]
[1]Chemistry Department, Imperial College, London SW2 2AY, U.K, [2]NIH, Bethesda, Maryland 20892, U.S.A.

In solution the structure of DNA does not conform to a straight ideal double helix. Besides structural distortions, thermally-induced bending, stretching and twisting fluctuations of DNA result in distortion away from the ideal structure. But, x-ray diffraction patterns of long DNA in aggregates are very close to those expected for ideal helices. The diffraction intensity is determined mostly by phosphates. Interactions between molecules in aggregates will favour an 'ordered' distribution of the charged phosphates in order to reduce the electrostatic energy. This will straighten the major axis of the molecule. Interactions dependent on helical structure will also reduce the distortions away from an ideal helix.Therefore, due to intermolecular interactions, the relative positions of each phosphate group will be different, when a molecule is isolated in isotropic solution as compared to when it is a constituent of a dense DNA assembly. We may quantity helix ideality through a helical coherence length, λ_c; the amount of distortion away from a perfect helix (an ideal helix has an infinite coherence length) [1]. In the theory of X-ray diffraction, λ_c determines the width of the layer lines. We examine real X-ray diffraction patterns of DNA fibres in order to estimate values of λ_c. We find that λ_c is larger than expected for molecules whose interaction can be approximated by uniformly charged rods. We conclude that helix-specific interactions play an important role in the structural adaptation of DNA, explaining the increase of the coherence length. [1]A. A. Kornyshev. D. J. Lee, S. Leikin, and A. Wynveen, Rev. Mod. Phys.(in press, 2007)

P-268

Structural determinants of the replication-block FapyG for Fpg specific recognition

Y.-V. Le Bihan[1], F. Coste[1], M. Ober[2], T. Carell[2], B. Castaing[1]
[1]Centre de Biophysique Moléculaire, Orléans, France, [2]Ludwig-Maximilians University, Munich, Germany

The Formamidopyrimidine-DNA glycosylase (Fpg) is a DNA repair enzyme which excises oxidized purines such as 7,8-dihydro-8-oxoguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) from damaged DNA. Here, we report the crystal structure of the wild type Fpg protein from *Lactococcus lactis* (*Ll*Fpg) bound to the substrate analogue *c*FapydG-containing DNA. The structure reveals that Fpg stabilizes the *c*FapydG nucleoside into an extrahelical conformation inside its substrate binding pocket. In contrast to the recognition of the 8-oxodG lesion which is bound with the glycosidic bond in a *syn* conformation, the *c*FapydG lesion displays in the complex an *anti* conformation. Furthermore, Fpg establishes interactions with all the functional groups of the FapyG base-lesion which can be classified in two categories: *(i)* those specifying a purine derived lesion (here a guanine), involved in the Watson-Crick face recognition of the lesion and probably contributing to an optimal orientation of the pyrimidine-ring moiety in the binding pocket and *(ii)* those specifying the imidazole-ring opened moiety of FapyG and probably participating also in the rotameric selection of the FapydG nucleobase. These interactions involve strictly conserved Fpg residues and structural water molecules mediated interactions. The significant differences between the Fpg recognition modes of 8-oxodG and FapydG provide new insights into the Fpg substrate specificity.

Abstracts

– Macromolecular assemblies –

P-269

Main features on bacteriophage T5 capsid assembly, DNA packaging and genome ejection

L. Letellier[1], L. Ponchon[1], A. Huet[1], P. Boulanger[1], G. Effantin[2], J. Conway[2]

[1]Institut de Biochimie Biophysique Moléculaire et cellulaire, CNRS, Unversité Paris Sud, Orsay, France, [2]University of Pittsburgh, USA

The *E.coli* phage T5 belongs to the Siphoviridae family that is characterized by a flexible and non contractile tail and an isometric icosahedral capsid. Several features make T5 an unusual and remarkable phage. Its genome (121kbp) carries genetically defined single-stranded interruptions and large (10160bp) terminal redundancies. DNA transport occurs by a unique two-step process (1). Sequencing of its genome (2) and proteome analysis have allowed identifying most of the genes encoding the structural proteins and the packaging machinery. The T5 structure was recently solved from cryo-electron microscopy images reconstruction allowing deciphering the capsid structure, the layered organization of the DNA within the capsid and the unusual trimeric organization of the tail (3).The proteins participating in assembly and maturation of the T5 capsid and those forming the molecular motor for DNA encapsidation (portal, terminase) (4) were overproduced, purified and are currently characterized. A model for DNA transfer to the host is proposed on the basis of real time imaging of DNA ejection from single phage particles (5).

(). Letellier,L et al. (2007) in " Bacteriophage Genetics and Molecular Biology" S.Mc Grath, ed. Horizon Press; (2) Boulanger, P. et al. Genbank AY692264 and AY734508; (3) Effantin, P.et al (2006) J. Mol. Biol. 361, 1003; (4) Ponchon, L. et al (2006) J. Biol. Chem. 281, 5829; (5) Mangenot, S. et al (2005) Current Biology, 15, 430.

P-270

Biomimic recognition and catalysis by an imprinted catalysts prepared by molecular self-assembly

S.-J. Li, D.-N. Zhang, W.-K. Li, P.-C. Chen

Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, China

Biomimic recognition and catalysis has been one of hot points in chemical research over the latest years. In the wake of a jump from combinatorial method to molecular assembly, the oriented design by crown ethers or cyptands toward molecular recognition is entering into an era of free imprinting. This article presents an original work contributing to the rational design of imprinted catalyst by molecular self-assembly toward predetermined high specificity. Assembling with *p*-nitrophenyl phosphate as the transition state analogue (TSA) of *p*-nitrophenyl acetate esterolysis and 1-vinylimidazole as the functional monomer, the imprinted catalyst was prepared. An increase in the amount of assembled monomer results in a higher activity of hydrolysis, which, however, does not lead to an improvement of specificity. The best specificity is shown at the optimal self-assembly (corresponding to a stoichiometric interaction of monomer-TSA). Higher or lower an amount of assembled monomer would lead to a dramatic decrease in this specificity. Related information indicates that these may be a result of increasing specific interaction between the TSA and binding sites, which make the catalyst capable of selectively recognizing the transition state and promoting the conversion from reactant to the transition state.

P-271

Rationally designing an imprinted antibody by metal mediator toward predetermined high selectivity

S.-J. Li, M. Zheng, C. Liao

College of Chemistry, Central China Normal University, Wuhan 430079, P. R. China

Biomimic recognition has been intensively studied over the past few years due to its importance on a broad range of applications. Recently, this field is significantly advanced by the so-called molecular imprinting. Normally, based on the self-assembly of functional monomers and template, the antibody is produced by a crosslinking polymerization. The template is then removed from the polymer, leaving behind binding sites complementary to the imprint molecule in terms of the shape and position. In this article, an original work contributing to rationally designing the imprinted antibody by metal mediator toward predetermined high selectivity was presented. Assembling with cobalt as the pivot, the imprinted antibody was obtained by arranging polymerizable monomers (Methacrylic acid) around a protein template (Boc-L-Phe-OH). The result indicates that the use of stoichiometric cobalt plays obviously a role on significantly increasing the molecular recognizability of imprinted antibody. A higher or lower amount of cobalt would lead to a dramatic decrease in this recognizability. Related information indicates that these, in logic, can be a result of the increasing matchablity between the template and binding sites, which makes the antibody capable of selectively recognizing the imprint species.

P-272

Electron tomography reveals the structure of the C-zone in striated muscle

P. K. Luther[1], H. Winkler[2], K. Taylor[4], R. Craig[3], R. Padron[4], M. Zoghbi[3], J. Liu[2]

[1]Imperial College London, UK, [2]FSU, Tallahassee, USA, [3]Univ of Mass Med Sch, Worcester, USA, [4]IVIC, Venezuela

The C-zone in vertebrate striated muscle is the region in each half A-band where myosin binding protein C (MyBP-C) forms at least 7 bands of 43 nm spacing. Mutations in cardiac MyBP-C are a major cause of familial hypertrophic cardiomyopathy; hence there is great interest in understanding the role of MyBP-C in the sarcomere. We show that the organisation of the C-zone is very similar in cardiac and skeletal muscles. We report on the 3D organisation of the C-zone by electron tomography of an exceptionally well-preserved frog skeletal muscle which shows prominent MyBP-C stripes. Thin $\sim$100nm sections were imaged using an FEI CM300 electron microscope to obtain three dual-axis tomograms. An average image of the thick filament was calculated from extracted cylindrical volumes that included single filaments and surrounding six actin filaments. The average thick filament shows similar structure within each 43 nm band in the C-zone, which comprises MyBP-C and 3 layers of myosin head crowns of separation $\sim$14.3 nm. Layer 1 is the very dense MyBP-C layer, while layers 2 and 3 are simple myosin head crowns. We show the results of fitting myosin heads to the three levels. What is intriguing about Layer 1 is that it comprises strong density sharply defined along a narrow axial zone. This layer comprises myosin heads and MyBP-C. Fitting of the myosin heads suggests that MyBP-C projects beyond the heads, reaching the actin filaments. We present a model for the arrangement of MyBP-C, and we speculate about the role of MyBP-C in the sarcomere.

Abstracts

– Macromolecular assemblies –

P-273

Structural basis for regulation of the 4-megadalton branched-chain α-ketoacid dehydrogenase complex

M. Machius[1], J. Li[1], J. L. Chuang[1], R. M. Wynn[2], D. T. Chuang[1]
[1]Dept. Biochemistry, [2]Dept. Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, U.S.A.

The human mitochondrial branched-chain α-ketoacid dehydrogenase complex (BCKDC) catalyzes the oxidative decarboxylation of branched-chain α-ketoacids for lipogenesis and energy production. This 4-MDa multienzyme complex contains a 24-meric transacylase (E2) core, with multiple copies of a decarboxylase (E1), a dehydrogenase (E3) and a kinase and phosphatase non-covalently attached. The lipoyl-bearing domain (LBD) of the E2 core channels reaction intermediates by sequentially visiting the active sites in the E1, E2 and E3 components. Regulation is achieved on several levels through intricate and surprising mechanisms. First, phosphorylation of only one of the two E1α subunits is sufficient to abolish overall BCKDC activity. To explain this puzzling observation, an alternating active-site mechanism for E1 was proposed. However, using crystallography, enzyme kinetics and mutagenesis, we now show that the two E1 active sites are independent. Instead, it is the geometry of the multienzyme assembly that restricts phosphorylation and catalytic activity to only half of the E1 active sites. Second, thiamin diphosphate-dependent E1 exists in a hyperactive state, controlled by the re-orientation of a tyrosine side chain during the formation of the decarboxylation intermediate. This localized structural change propagates to the surface of E1 and initiates LBD binding to extract the intermediate for delivery to the E2 active site. This signaling event establishes a strict order for the four distinct chemical reactions carried out by BCKDC. Our study is a prime example for the complex regulatory mechanisms in a large multienzyme assembly.

P-274

Assembly and dynamics of photosynthetic membranes in Purple Bacteria.

C. Mascle-Allemand[1], S. Scheuring[2], V. Prima[1], J. N. Sturgis[1]
[1]LISM UPR9027 and Université de la Mediterreanée, IBSM, Marseille France, [2]Institut Curie, Paris, France

Purple bacteria have a particularly simple photosynthetic system involving 5 membrane embedded components: the light harvesting complex LH2; a reaction center containing core complex; a ubiquinone pool; a cytochrome bc_1 complex and a F_0F_1 ATP synthase. These components are assembled in specialized membranes, often contiguous with the cytoplasmic membrane. Recently we have investigated the organization, function and dynamics of these components in several bacterial species and growth conditions. These studies have relied on AFM, spectroscopic measurements and modeling.

Analysis of the observed organization suggests a hierarchy of different intramolecular forces leading to the formation of membrane domains of specific composition at several different scales. We find that assembly of these specialized membranes depends in part on long-range non-specific interactions, leading to poor solubility of the proteins in the membrane. The resulting very protein dense organization if observed to have very limited dynamics, nevertheless if appears that the organization paradoxically could result in the acceleration of electron transfer between the reaction center and the cytochrome bc_1 complex. Inter-specific comparisons suggest that different species have chosen different methods for organizing their photosynthetic apparatus.

We discuss these results in terms of the different strategies observed for optimizing photosynthetic efficiency in these organisms.

P-275

Room temperature phosphorescence of human erythrocyte membranes: action of protein cross-linking agents

V. M. Mazhul´, I. V. Halets
Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, Minsk, Belarus

Room temperature tryptophan phosphorescence measurements were used to report on the influence of intra- and intermolecular cross-links on intenal dynamics of proteins of isolated human erythrocyte membranes *in situ*. Erythrocyte membranes were reacted with a series of different protein cross-linking reagents, addressing to the different protein's groups and forming linkages of different length. We used bifunctional reagents – glutaraldehyde, dimethyl suberimidate and condensing carbodiimides – 1-ethyl-3(3-dimethylaminopropyl)-carbodiimid hydrochloride, dicyclohexylcarbodiimid in our experiments. Time resolved phosphorescence measurements of samples and decay analysis were performed using home-made instrumentation and methods [1].

It has been shown that treatment of erythrocyte membranes by both bifunctional and condensing cross-linking reagents in millimolar concentrations results in considerable (up to several folds) increase phosphorescence lifetime. The data indicate the restriction of internal dynamics of membrane proteins in the vicinity of tryptophan residues. The shape of dependence of membranes phosphorescence lifetime on the cross-linking reagent concentration turned out to be specific for every reagent.

Acknowledgement:
This work is supported by ISTC, Grant B-1332

References:

[1]V.M. Mazhul', E.M. Zaitseva, D.G. Shcherbin. Biophysics, **46** (2000), 935–959

P-276

Using well-defined peptide model systems to study membrane proteins

T. Meijneke[1], T. K. M. Nyholm[1], D. T. S. Rijkers[2], R. M. Liskamp[2], A. J. Killian[1]
[1]biochemistry of Membranes, Utrecht University, The Netherlands, [2]Medicinal Chemistry and Chemical Biology, Utrecht university, The Netherlands

The structure and activity of membrane proteins can be significantly influenced by lipids. To attain knowledge on the general principles of such protein-lipid interactions simple model systems can be used, consisting of synthetic peptides in lipid vesicles. In these systems, properties of both peptide and lipids can be systematically varied. Examples are the WALP peptides, which consist of a sequence of alternating alanine and leucine, flanked on both sides by tryptophan residues. These peptides incorporate in membranes as well-defined transmembrane helices that can be characterized by various biophysical methods. However, most proteins have multiple membrane spanning segments. To better mimic this situation, we synthesized three different cysteine-containing WALP analogs that can be oxidized to form covalent dimers. The cysteines are all at approximately the same depth in the middle of the membrane, but at a different face of the helix. In addition tetrameric assemblies of peptides are being synthesized. WALP-peptides have been designed that can be coupled to a scaffold via click-chemistry. The influence of oligomerization on the properties of the helices and on their interaction with the lipids can than be studied by for example 2H-NMR on peptides with deuterated alanines or on lipids with deuterated acyl chains. In addition, with the cysteine containing WALP peptides dimerization experiments are being performed to find out if there is a preferential helix-helix interface.

Abstracts

– Macromolecular assemblies –

P-277

Self-assembly of centralspindlin, a protein complex of mitotic kinesin and RhoGAP

M. Mishima[1], M. Glotzer[2]

[1]Wellcome/CRUK Gurdon Institute, University of Cambridge, UK, [2]Deptartment of Molecular Genetics and Cell Biology, University of Chicago, Illinois, U.S.A.

Centralspindlin is a conserved protein complex, containing kinesin and RhoGAP subunits, that is essential for the formation of the central spindle and midbody, microtubule-bundle structures critical for cytokinesis. We previously reported that centralspindlin oligomerizes under physiological conditions though the biological significance of this oligomerization was unclear. Here, we show that the kinesin subunit ZEN-4 contains a small region at the C-terminal end of its predicted coiled coil that is essential for self-assembly of centralspindlin. Mutant versions of ZEN-4 specifically deficient in self-assembly by deletion of this region were defective in microtubule-organization in vitro. Self-assembly-deficient *zen-4* transgenes rescued *zen-4* null animals less efficiently than wild type *zen-4* transgene. The mutant ZEN-4 failed to accumulate to the midbodies, leading to the abortive cytokinesis. These data indicate that the biological function of a kinesin motor requires formation of a large polyvalent motor complex akin to the myosin minifilament.

P-278

Quantitative characterization of hydration and volumetric changes in protein folding

L. Mitra[1], K. Hata[2], R. Kono[2], A. Maeno[2], D. Isom[3], K. Akasaka[2], R. Winter[1], B. Garcei-Moreno E.[4], C. A. Royer[4]

[1]University of Dortmund Department of Physical Chemistry, Germany, [2]Kinki University Department of Biotechnology, Higashi-Osaka City, Japan, [3]The Johns Hopkins University Department of Biophysics, Baltimore, U.S.A., [4]Inserm, U554, Centre de Biochimie Structurale, Montpellier, France

The exquisite specificity of protein structure, dynamics and function is intimately linked to interactions with water. Pressure studies should, in principle, provide insight into these changes in hydration and the energetic and structural role of the solvent in determining the energy landscape of proteins. However, understanding the role of solvent in protein conformational transitions has been hindered due to a lack of a fundamental and quantitative understanding of volumetric properties of proteins. We provide a quantitative characterization of the volumetric properties of a model protein (Snase) and demonstrate how subtle conformational changes can modulate these properties. These results underscore the complexity of volumetric properties of proteins and demonstrate that their interpretation in terms of solvation must be made in the context of their complete temperature dependence. Finally, by testing the effects of insertion of ionisable residues at various positions throughout the Snase structure, we have mapped using p-jump the structure of the transition state ensemble of Snase. Our results show that the β-barrel and abutting helix is well structured in the TSE, that the linker region between this subdomain and the terminal helix is partially structured, and that the C-terminal helix remains unordered in the Snase TSE.

P-279

Pressing over tubulin

S. Monnier[3], C. Larroque[1], N. Bec[1], A. Parmeggiani[2], M. Stephane[4], L. Reinhard[4], V. Lorman[3]

[1]CRLC Val d´Aurelle, INSERM, Montpellier, France, [2]DIMNP,UMR 5235 CNRS/UM2, Montpellier, France, [3]LPTA, UMR 5207 CNRS/UM2, Montpellier, France, [4]U710, INSERM, Montpellier, France

Microtubules (MTs) are major constituents of the cytoskeleton playing a fundamental role for cellular functions like intracellular transport and mitosis. MTs are, therefore, among the most sensitive targets for drugs used in chemotherapy of cancers. These cylindrical structure of tubulin dimers (TDs) are characterized by a unique non-equilibrium assembly-disassembly so called "dynamic instability". In cells any perturbation of this dynamics is reported to cell cycle "check points" and engages the cell apoptotic pathway. In this work, by using spectroscopy techniques and electron microscopy, we studied how a physical perturbation such as hydrostatic pressure affects dimer conformation as well as TDs assembly dynamics. We found that, for typical regime of TDs polymerization in vitro, hydrostatic pressure can drive an irreversible conformational change of the TD, affecting both TD assembly topology and dynamics. Significant variation of conformation kinetics as a function of applied pressure was also observed. Moreover, experiments suggest that even a low pressure (∼50 bars) can affect directly the assembly dynamics and structure even in absence of significant conformational changes of the TD as observed at higher pressure (>400 bars). To compare the pressure influence on the TD and MT assembly with the action of classical agents, we also studied the role of temperature changes as well as the biochemical action of anti-tumoral drugs like taxol.

O-280

A unique mechanism of microtubule stabilisation by doublecortin

C. Moores[1], M. Perderiset[2], C. Kappeler[3], S. Kain[4], D. Drummond[4], J. Chelly[3], R. Cross[4], A. M. Houdusse[2], F. Francis[3]

[1]Birkbeck College, London, UK, [2]Institut Curie CNRS, Paris, France, [3]Institut Cochin, Paris, France, [4]Marie Curie Research Institute, Oxted, UK

Neurons undertake an amazing journey from the centre of the developing brain to the outer layers of the cerebral cortex. Many cellular factors are essential for this cellular migration, including the cytoskeleton. Doublecortin, a component of the microtubule cytoskeleton, is essential in postmitotic neurons and was identified because its mutation disrupts human brain development. Doublecortin stabilises microtubules but has no homology with other microtubule associated proteins (MAPs). We used electron microscopy to visualise the doublecortin binding site on microtubules, which is unique among MAPs and confers unusual properties on doublecortin. We have characterised these properties biophysically to help understand how doublecortin influences microtubule dynamics and thereby brain development. Doublecortin has no effect on microtubule growth rate but it stabilises microtubules against depolymerisation. Doublecortin also nucleates microtubules. In cells, doublecortin localises to the distal ends of neuronal processes and our data raise the possibility that the function of doublecortin in neurons is to drive assembly and stabilisation of non-centrosomal microtubules in these doublecortin-enriched distal zones. These distinct properties combine to give doublecortin a unique function, a role that cannot be compensated for by other microtubule stabilising proteins and nucleating factors.

Abstracts

– Macromolecular assemblies –

P-281

Hierarchic finite level energy landscape model of phosphoglycerate kinase refolding

S. Osváth[1], L. Herényi[1], P. Závodszky[2], G. Köhler[3], J. Fidy[1]
[1]Department of Biophysics and Radiation Biology, Semmelweis University, Puskin u. 9, H-1088 Budapest, Hungary, [2]Institute of Enzymology, Biological Research Center of Szeged, Karolina u. 29, H-1113 Budapest, Hungary, [3]Max F. Perutz Laboratories, Department of Chemistry, University of Vienna, Rennweg 95B, A-1090 Vienna, Austria

One of the most intriguing predictions of energy landscape models is the existence of non-exponential protein folding kinetics caused by hierarchical structures in the landscapes. Here we present a hierarchic landscape model to describe folding of yeast phosphoglycerate kinase. Refolding was initiated from the guanidine-unfolded state by stopped-flow or manual mixing, and monitored by tryptophan fluorescence from 1 ms to 15 minutes. The strategy to build a model that describes folding of yeast phosphoglycerate kinase was to start from the simplest paradigm and modify it stepwise to the necessary minimal extent after repeated comparisons with the experiments. We made no a priori assumptions about the folding landscape. The result was a Hierarchic Finite Level landscape model that quantitatively describes the refolding of yeast phosphoglycerate kinase. The early steps of the folding process happen in the upper region of the landscape, where the surface has a hierarchic structure. This leads to stretched kinetics in the early phase of the folding. The lower region of the energy landscape is dominated by a trap that reflects the accumulation of molten globule intermediate state. From this intermediate, the protein can reach the global energy minimum corresponding to the native state through a cross-barrier folding step.

O-282

Lipase-specific foldases: steric chaperones as dynamic folding platforms

K. Pauwels[1], D. Bourry[2], A. Dubus[3], J. Martins[2], E. de Pauw[3], L. Wyns[1], P. van Gelder[1]
[1]Department of Molecular and Cellular Interactions, VIB & VUB, Brussels, Belgium, [2]NMR en Structuuranalyse, Ugent, Ghent, Belgium, [3]Mass Spectrometry Laboratory, ULg, Liege, Belgium

In Gram-negative bacteria the decisive step in the biogenesis of type II secreted proteins comprises their periplasmic folding. These proteins are exported over the inner membrane via the Sec- or Tat-machinery and then become translocated over the outer membrane by a macromolecular complex that spans the entire cell envelope. However, the transient folding of the secretion cargo, which relies on very specific chaperones, is a prerequisite for recognition by the secretion machinery. The lipase (LipA) of *Burkholderia glumae* depends on a lipase-specific foldase (Lif) to fold completely into its native, active and secretion competent conformation. The crystal structure of the soluble form of the *B. glumae* Lif in complex with LipA is the first molecular snapshot of a Lif. The periplasmic domain of Lif consists of 11 alpha-helices that are wrapped around LipA. All aromatic residues are located in two hydrophobic clusters that are located at the distal ends of the visible structure. Our structure is complemented by a biophysical approach comprising fluorescence and CD spectroscopy, NMR, chemically induced denaturation, limited proteolysis and mass spectrometry. Our studies suggest that Lif undergoes a coil-helix conformational transition upon exerting its function. Lif thus represents a novel type of steric chaperone that forms a dynamic folding platform to accommodate the complex folding behaviour of its target lipase.

P-283

A step toward the reconstitution of a cell cortex

L.-L. Pontani[1], J. van der Gucht[2], C. Sykes[1]
[1]Laboratoire Physico-Chimie Curie, Unité Mixte de Recherche 168, Institut Curie, 11 rue Pierre et Marie Curie, 75231 Paris, Cedex 5, France, [2]Laboratory of Physical Chemistry and Colloid Science, Wageningen University, 6701 BH Wageningen, Netherlands

Actin assembly at the plasma membrane is the driving force for cell deformation and movement, while an actin cortex in conjunction with myosin motors provides sufficient contractility to generate shape changes of the cell. We develop a biomimetic approach for a better understanding of cell cortex dynamics, as follows. The necessary ingredients for actin polymerization are introduced into a liposome. Polymerisation at the membrane, as opposed to in the bulk of the vesicle, is performed by grafting actin polymerization activators to the bilayer through histidine nickel interactions. By similar methods using an inside out geometry, actin comets were observed on liposomes coated with nucleators, confirming the possibility of actin polymerisation on such a bilayer. Polymerisation is then triggered inside the liposome and an actin cortex is successfully observed at the membrane. The next step is to introduce myosin motors into this cortex to study the effects of contractility on vesicle shape.

P-284

Biophysical studies on biodegradable triblock copolymers

P. S. Pourhosseini, A. A. Saboury, A. Divsalar, M. N. Sarbolouki
Institute of Biochemistry and Biophysics, University of Tehran, PO Box 13145-1384, Tehran, Iran

This work aims at evaluating the aqueous dispersion of triblock copolymers as potential drug delivery candidates. Hereby the interaction of oligoanhydride-PEG triblock copolymer with bovine insulin as a model protein is extensively investigated by isothermal titration calorimetry (ITC) and fluorescence spectroscopy. ITC experiments show that the copolymer has low affinity for the protein, demonstrating a fairly weak interaction. Results of fluorescence spectroscopy also indicate that the protein structure becomes slightly more flexible in the presence of copolymer. Altogether, these observations indicate that the copolymer slightly alters the protein structure.
Furthermore, this study demonstrates that hydrophilic compounds can be readily encapsulated inside polymersomes made of these triblock copolymers. Extensive studies on the release properties of polymersomes, both at room temperature and at 37° C show a first order kinetics, indicating that the polymersome system acts as a controlled membraneous reservoir release system. Experiments on determination of critical aggregation concentration (c.a.c.) as well as zeta potential of these polymeric vesicles are currently under investigation and will be reported.

Abstracts

– Macromolecular assemblies –

O-285

Subunit Architecture of protein complexes derived from mass spectrometry

C. Robinson
Department of Chemistry, University of Cambridge, Cambridge, UK

High throughput proteomic approaches have identified numerous *in vivo* protein complexes and suggested the existence of many others from extensive protein interaction networks. Standard proteomics techniques are unable to describe the stoichiometry, subunit interactions and organisation of these assemblies since many are heterogeneous, present at low cellular abundance and frequently difficult to isolate. We have combined two existing methodologies to tackle these challenges: tandem affinity purification (TAP) and nanoflow ESI-MS. Our rationale is based on the selectivity offered by TAP for isolation of complexes at natural expression levels and the direct detection of complexes achievable by nanoflow ESI-MS. We use methods designed to maintain non-covalent complexes within the mass spectrometer to provide definitive evidence of interacting subunits based on the masses of complexes and subcomplexes generated by perturbation both in solution and gas phases. Data will be presented for three oligomeric protein complexes, the yeast exosome (1) and the proteasome lid complex (2) and human eIF3 complex, each containing in excess of nine subunits.

References
1.Hernandez H, Dziembowski A, Taverner T, Seraphin B, Robinson CV. 2006. *EMBO Rep* 7: 605-10
2.Sharon M, Taverner T, Ambroggio XI, Deshaies RJ, Robinson CV. 2006. *PLoS Biol* 4: 1314-23

P-286

Insights into the stability of an extremophilic protease complex

E. Rosenbaum[1], A. Durá[1], F. Gabel[1], F. Vellieux[1], P. Masson[2], G. Zaccai[3], B. Franzetti[1]
[1]Institut de Biologie Structurale, Grenoble, France, [2]CRSSA, La Tronche, France, [3]Institut Laue-Langevin, Grenoble, France

TET3 is an aminopeptidase characterized in the hyperthermophilic archaea *Pyrococcus horikoshii*. Enzymatic studies have shown that TET3 is an N-terminal exopeptidase, which means that it degrades polypeptides by cleaving the amino acid at the N-terminal. It is optimally active at 85°C and forms large tetrahedral homo-oligomeric dodecamers. However it is still not known, if complexes of this size can exist in the extreme physico-chemical conditions to which extremophilic organisms are exposed. The existence of cavities inside the protein should further destabilize the complex.Therefore it is possible that the TET3 dodecamer will undergo structural and conformational changes in extreme conditions.

To elucidate this question, we have solved the structure of TET3 by x-ray crystallography and we have studied the oligomerization of TET3 under different extreme conditions by analytical ultracentrifugation (AUC), small angle neutron scattering (SANS) and small angle x-ray scattering (SAXS).

The complex has a diameter of about 130Å. The active sites are located inside the complex and are accessible by a system of channels. Thus the active sites are shielded away from the cytoplasm, preventing unspecific peptide degradation.

Our in-solution studies show that TET3 can exist in a large variety of extreme conditions, amongst others high temperature, high and low salt and high and low pH.

P-287

Structural studies on P-glycoprotein

C. A. Shintre[1], C. A. McDevitt[2], S. M. Prince[1], R. C. Ford[1], R. Callaghan[2]
[1]University of Manchester, [2]Univeristy of Oxford

P-glycoprotein is a member of a large family of membrane spanning proteins called ABC transporters [ATP (Adenosine tri-phosphate) Binding Cassette] which transport solutes across the cell membrane in an ATP dependent manner. Over the past three decades a vast amount of research has been done to understand their mechanism as they play important roles in biological phenomena such as multidrug resistance observed in cancer and bacterial pathogens. There is a growing link between the expression of P-glycoprotein and the ineffectualness of chemotherapy. The basic structure consists of two trans-membrane domains (TMDs) mainly composed of alpha helices and two nucleotide binding domains (NBDs) which bind the nucleotide to power the reaction. The precise events of the catalytic cycle are still unknown.

This project focuses on methods to probe the structure of P-glycoprotein using X-ray crystallography and electron microscopy. Electron crystallography as well as single particle analysis has been used in the past to generate a low resolution structure for P-gp.

The use of detergents which is essential in membrane protein solublisation adds an additional dimension to the procedure of crystallization. Here, initial conditions were firstly screened with commercially available sparse-matrix screens. Optimization for temperature, secondary detergents, additive salts and small molecules as well as pH was then carried out. Electron microscopy was used to assess the homogeneity of the protein using single particle analysis. Various nucleotide analogs and drugs which act as substrates for P-gp were tested to take a more rational approach to crystallization.

P-288

Copolymerization of biomolecules and polymers grafted with Silicon: Application to Biosensors, Biochips and development to bioelectronics.

S. Singhal, M. Singh
Chemistry Research Lab, Deshbandhu College, University of Delhi, New Delhi, 110019, India

The area of biosensor development is becoming increasingly hi-tech. From the literature, the properties of amino acids, polylactamides (PVP) and silicon are reported conducting. In the author's laboratory, attempts have been made to develop simple and biocompatible biosensor using sol-gel technique. In general, the alpha amino acids and polylactamides are converted into their stable derivatives and then are introduced into the steps of the hydrolysis of TEOS under controlled conditions. The product obtained are characterized using spectroscopic techniques namely IR and NMR and stability is measured with thermal analysis i.e. TGA/DTA. These metal-based copolymers are assumed to have controlled conducting properties, which are in high demands for defense purposes. Several chemical moieties present in copolymer enables it to fascinitate in nanotechnological innovations, especially weakly conducting synthetic biopolymers due to their tremendous applications in biochips, biophysics and drug delivery systems. The copolymer is supposed to be biocompatible as it has amino acid in its structural framework and also conducting material due to silicon.

Abstracts

– Macromolecular assemblies –

P-289

Pore Formation of Insecticidal Cry4B Toxin in Phospholipid Bilayers: Atomistic Simulation Studies

I. Sramala[1], C. Angsuthanasombat[2]

[1]National Nanotechnology Center, NSTDA, Patumthani, 12120, Thailand, [2]Laboratory of Molecular Biophysics, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom, 73170, Thailand

It has been demonstrated that a hairpin fragment of two transmembrane helices (alpha-4-alpha-5) of the 130-kDa *Bacillus thuringiensis* Cry4B toxin is responsible for its ion channel formation and mosquito-larvicidal activity. Assembling of the ion channel was characterized by means of nanosecond (ns) molecular dynamics (MD). The simulation started with assumed states of oligomeric peptides in preformed lipid bilayer system (POPC/water). After 5 ns equilibration of unrestrained MD, the most energetic stable structure was used as the final structure for further calculations. Two steps of pore formation mechanism were explored. (A) Pore forming fragment was positioned on the surface of equilibrated phospholipid membrane. The fragment was then forced into the bilayers. To allow developing of a proper orientation, peptide was pulled into the lipid membrane at velocity of 0.1 m/s. (B) Pore forming fragments were pre-inserted into equilibrated lipid bilayers with intermolecular distance of 10 Angstroms. The fragments were then subjected to MD simulations under restrains of the final tethered structure.
Similar studies were also performed by using dimeric peptide as the initial structure. Higher degree of energetic favorability was observed and discussed. It was also suggested that the spontaneous ion channel assembly of insecticidal peptides in phospholipid bilayers is likely initiated by oligomeric complex of the pore forming fragment.

P-290

Hydrogen-deuterium exchange revealed dynamics and interactions in a dsRNA bacteriophage

B. Suchanova[1], V. Manole[1], S. Kang[2], L. Happonen[1], A. Ora[1], P. E. Prevelige[2], S. J. Butcher[1], R. Tuma[1]

[1]Institute of Biotechnology, University of Helsinki, Finland, [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, USA

Icosahedral dsRNA bacteriophages from *Cystoviridae*family represent excellent model systems for assembly of other dsRNA viruses such as members of *Reoviridae*family. Cystoviruses assemble in two stages, making an empty polymerase complex (PC) first into which RNA precursors are subsequently being packaged by a viral packaging motor. The packaged RNA is then replicated inside PC by an associated RNA polymerase. These processes are regulated by a sequence of conformational changes in the major structural protein P1. Thus, PC is a relatively simple example of molecular machine and we are interested in delineating the mechanisms involved in its assembly and function.
This study is focused on dynamics and interactions of the P1 protein within the PC assembly. We applied hydrogen deuterium exchange detected by high-resolution mass spectrometry. This method allowed to resolve kinetics of region-specific exchange and map them onto the primary structure. We are currently developing new bioinformatics tools to exploit the exchange kinetics in the context of medium resolution electron density map obtained by cryo-microscopy. This will allow delineation of subunit interfaces in the absence of high-resolution structure of P1 and at the same time extend the interpretations of cryo-microscopy results.

P-291

Acetylation induced chromatin compaction is histone specific

K. Toth, M. Bussiek, A. Gansen, J. Langowski

German Cancer Research Center, Div. Biophysics of Macromolecules (B040), Heidelberg, Germany

Histone acetylation is known to be connected to increased gene activation and hyperacetylation correlates sometimes with cancer. The structural base of this correlation is supposed to be local chromatin decompaction due to weakened interaction between neutralized histone tails and linker DNA. Using fluorescence resonance energy transfer (FRET) we analyzed changes of the linker DNA path on reconstituted mono- and oligonucleosomes with differently acetylated recombinant histones. On trinucleosomes the compaction was also imaged by scanning force microscopy (SFM). Both mono- and trinucleosomes show that the compaction effect is histone-specific. Acetylation of all histones or H3 histone alone decompacts the structure as measured by increase in the linker DNA distances, in the internucleosomal angles and in the internucleosomal distances. Acetylation of H4 histone alone leads on the contrary to compaction of the structure. This result harmonizes with earlier observations on correlation between gene activation and acetylation of H3 or deacetylation of H4 histones. Single molecule measurements (spFRET) are undertaken to elucidate further structural details.

P-292

Interaction of oligonucleotides containing LNA with preformed cationic lipid vesicles

T. Vissing

Genetics & Bioinformatics, Faculty of Life Science, Univ. Copenhagen, Denmark

The present study examines the interaction and entrapment of oligonucleotides containing Locked Nucleic Acids (LNA) in spontaneously formed cationic liposomes.
The oligonucleotides (ODN) shared the same sequence, with segments of LNA at various positions and representing different backbone nucleic acid chemistries. The lipid vehicles containing cationic lipids and polymeric coating, are suitable for straightforward entrapment of oligonucleotides at high loading ratios, forming lipid vesicles The resulting ODN-LNA entrapped liposomal system consisted of particles of sizes somewhat larger than parent LUV's.
Estimation was done of the average size and dispersity of the ODN-LNA loaded lipid vesicles, using both FACS and dynamic light scattering. Indications were that the thiolated backbone seems to promote slightly smaller lipid assemblies.
It was also revealed that the LNA gap-mers used in this study, showed that alpha-L-LNA promote particles more dense in ODN's. The scattering as observed by FACS, were different when comparing the LNA-phosphodiester with the phosphorothioate chemistry.
Results indicate that LNA containing ODN's can be loaded fairly efficiently and characterized such that they may become a tool for comparative studies of antisense oligonucleotides. This may be considered for the optimization of ODN delivery in cellular in-vivo and in-vitro uptake studies.

Abstracts

– Macromolecular assemblies –

P-293

Packing of monodisperse DNA-RecA protein complexes

T. Vuletic, E. Raspaud, A. Leforestier, M. Renouard, F. Livolant
Laboratoire de Physique des Solides, Universite Paris XI, Orsay, France

We report on a study in progress of *E.coli* RecA protein, from preparation and purification of protein monomers to structural studies of protein polymers formed on DNA – nucleoprotein filaments. This protein is a multirole one, where DNA strand exchange by forming nucleoprotein filament during homologous recombination and cleavage of SOS response repressors are the most prominent two. The first role, where we are interested into structural details (and structure makes function, very much so in this case) also came recently into focus with the work of Zahradka et al., (Nature 2006), where reassembly of shattered chromosomes in *D. radiodurans* is completed by RecA-dependent crossovers.
RecA is a relatively small protein, MW = 37,842, and only by polymerizing within nucleoprotein filaments it achieves its function. The RecA polymers have been crystallized and structure determined to atomic resolution by X-ray diffraction, however the structure of RecA-DNA complex has not been solved and the exact path of DNA within the nucleoprotein filament is not known. We are producing dense phases of RecA nucleoprotein filaments using very short, monodisperse, 146 bp long DNA. Such 50∼75 nm long filaments are shorter than their respective persistence length – *i.e.* we expect they will behave as straight rods. Presumably, an ordered nucleoprotein filament phase, studied by optical and electronic microscopies and XRD will allow further insight into the function of RecA, especially if a correlation will be found between the length of the filaments and their activity within the dense phase.
* *permanent position*: Institut za fiziku, Zagreb, Croatia

P-294

DISCO a synchrotron beamline dedicated to biophysics

F. Wien, A. Giuliani, M. Refregiers
Soleil Synchrotron - L´Orme des Merisiers - St. Aubin - France

DISCO a synchrotron beamline covering spectral ranges between 60 nm to 800 nm. Dedicated to biology and chemistry, it exceeds conventional laboratory light sources in terms of flux, brilliance and intensity. Beginning 2008 three experimental stations will be installed with following objectives:
Circular Dichroism: For the determination of secondary structures of soluble and membrane proteins [1], nucleotides (DNA, RNA) in solutions. Characterisation of protein–protein, protein-ligand and protein-nucleic acid interactions.
Imaging: Observations of inter and intra cellular biochemical reactions in living cells; Drug distribution analysis within human tissues, cancer cell and tissue studies by auto- fluorescence [2].
Mass Spectrometry: Identification of biological and chemical fragments induced by atmospheric pressure photo-ionisation (APPI) coupled to a mass spectrometer (Q-TOF) [3].
[1] Wien, F., Miles, A. J., Lees, J. G., Cuff, A. L., Janes, R. W., & Wallace, B. A. (2005). A new circular dichroism spectroscopy reference dataset covering fold space. Biophys. J. 88, 554A.
[2] Kocanova, S., Hornakova, T., Hritz, J., Jancura, D., Chorvat, D., Mateasik, A., Ulicny, J., Refregiers, M., Maurizot, J. C., & Miskovsky, P. (2006). Characterization of the interaction of hypericin with protein kinase c in U-87 MG human glioma cells. Photochem. Photobiol. 82, 720-728.
[2] Debois, D., Giuliani, A., & Laprevote, O. (2006). Fragmentation induced in atmospheric pressure photoionization of peptides. J. Mass Spectrom. 12, 189-197.

P-295

Structures of the TF1-ATPase ε subunit suggesting ATP-regulated arm motion

H. Yagi[1], N. Kajiwara[1], H. Tanaka[1], T. Tsukihara[1], Y. Yamada[2], M. Yoshida[3], H. Akutsu[1]
[1]Institute for Protein Research, Osaka University, Japan, [2]College of Science, Rikkyo University, Japan, [3]Chemical Resources Laboratory, Tokyo Institute of Technology, Japan

The ε subunit of bacterial and chloroplast F_oF_1-ATP synthases modulates their ATP hydrolysis activity. Here, we report the crystal structure of the ATP-bound ε subunit from a thermophilic *Bacillus* PS3 at 1.9 Å resolution. The C-terminal two α-helices were folded into a hairpin, sitting on the β sandwich structure, as reported for *E. coli*. A novel ATP binding motif, I(L)DXXRA, recognizes ATP together with three arginine and one glutamate residues. The *E. coli* ε subunit binds ATP in a similar manner, as judged on NMR. We also determined solution structures of the C-terminal domain of the PS3 ε subunit, and relaxation parameters of the whole molecule by NMR. The two helices fold into a hairpin in the presence of ATP but extend in the absence of ATP. The latter structure has more helical regions and is much more flexible than the former. These results suggest that the ε C-terminal domain can undergo an arm-like motion in response to an ATP concentration change and thereby contribute to regulation of F_oF_1-ATP synthase.

P-296

Coding sequences lengths of Escherichia Coli and Bacillus Subtilis by Detrended Fluctuation Analysis

O. Zainea[1], V. V. Morariu[1], C. Vamos[2]
[1]National Institute for R&D of Isotopic and Molecular Technologies, Cluj-Napoca, Romania, [2]T. Popoviciu Institute of Numerical Analysis, Romanian Academy, Cluj-Napoca, Romania

The lengths of coding sequences along the minus strand, plus strand and entire genome for the microbial genome of seven *Escherichia Coli* strains and *Bacillus Subtilis* strain *168* are investigated. Each of these strands is divided into four quarters and then Detrended Fluctuation Analysis (DFA) is applied. We find short-range correlation of coding sequence lengths. We get dissimilar values for the scaling exponent α corresponding to the chopped segments of minus, plus and both strands, in the range of 0.10-0.99, from anti-correlated to almost fractal-like fluctuations. Also, the DFA plot offers particular information in terms of α for each strain of the same organism and also for each investigated species - *Escherichia Coli* and *Bacillus Subtilis*.

Abstracts

– Macromolecular assemblies –

P-297

Transcription activation via σ^{54} activators

X. Zhang[1], M. Rappas[1], D. Bose[1], T. Pape[1], J. Schumacher[2], N. Joly[2], S. Wigneshweraraj[3], P. Burrows[2], M. Buck[2]
[1]Division of Molecular Bioscences, [2]Division of Biology, [3]Division of Investigative Sciences, Imperial College London, U.K.

Bacterial transcription relies on RNA polymerase (RNAP) dissociable sigma (σ) factors for promoter specificity. The major variant sigma factor (σ^{54}) forms a stable closed complex with RNAP on promoter DNA that rarely spontaneously isomerises to a transcriptionally-proficient open complex. Instead, ATP hydrolysis by bacterial Enhancer-Binding Proteins (bEBPs) bound to the upstream activating sequence (UAS) is required to remodel the RNAP-σ^{54}-DNA closed complex to the open complex conformation. bEBPs belong to the large AAA+ (ATPase associated with various cellular activities) protein family. Our recent work combining structural biology with functional characterisation of one type of bEBP, the *Escherichia coli* phage shock protein F (PspF), has provided atomic details on this activator in complex with different nucleotides as well as a structural model of PspF in complex with σ^{54}. Our results establish that PspF exists as a hexamer when in complex with σ^{54}, and by inference with the organisation of the RNAP/sigma54 holoenzyme. Our current studies focus on the mechanism by which PspF remodels its target – the RNAP σ^{54}holoenzyme closed complex. We present structural data on the RNAP/σ^{54} holoenzyme as well as the RNAP/σ^{54}/activator complex, which together provide an insight into the distinct functionality of the RNAP/σ^{54} holoenzyme and the mechanism of gene activation via bEBPs.

P-298

System logic

Q. Zhao
Medical institute, CRRC, POB 2619, Beijing 100068, PR China, Email: qyzh@163bj.com, website:www.zhaoqinyi.com

The biological organs, including protein machine, are complex system. However, the mathematics logic that can handle with complex system is still lacking. In the study of protein folding and protein dynamic, new mathematic logic (system logic) has been developed. In views of mathematics logic, a logical structure of condition/result characterizes a system, which shows cooperation phenomenon in view of biophysics. This structure reveals the fundamental and logic relation between structure and function of a system. The system logic can be deduced from this definition. It provides essential thinking tool in our study of protein dynamics, biosignal network (system), and other complex systems. The system logic is compatible with irreversible thermodynamics, synergetic, catastrophe, and etc. The system logic can transform into conventional mathematic logic (or element logic established by Aristotle in his studying of cause/result relation) under specific cases. As a conclusion, the axiomatic theory of biophysics, as well as biology, cannot be established based upon conventional logic. Therefore, conventional biophysics can only develop on the way of experimental science.

P-299

A Molecular Dynamics Study of Structural Organization in the Sphingomyelin - Cholesterol Bilayer

J. Zidar[1], F. Merzel[1], K. Sepcic[2], M. Hodoscek[1], K. Rebolj[2], M. Peter[2], D. Janezic[1]
[1]National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia, [2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, SI-1001 Ljubljana, Slovenia

It is well known that membrane composition is crucial in the formation of lipid membrane domains that form when an area of the membrane re-orders into so called liquid ordered phase. Such areas are important in the formation of raft-like domains, that are crucial for binding of biological ligands such as hormones, enzymes, etc.
One possible ligand is ostreolysin, a cholesterol-dependant citolysine, that binds lipid membrane only when the cholesterol molar ratio is above 30 % and the membrane lipids are properly ordered. To investigate the mechanism of lipid membrane reordering we constructed five lipid bilayers with 20 %, 30 %, 35 %, 40 % and 50 molar % of cholesterol, the remaining part was composed of sphingomieline. On all five systems we performed all - atom molecular dynamics simulations with fixed and relaxed size of the unit cell. Membrane systems were analysed after both steps in order to find possible structural differences cause by the simulations.
Our results indeed indicate structural changes in membrane ordering happening during simulation, where greater changes are observed in simulations with relaxed unit cell. The greatest structural differences between the systems were clearly observed when the cholesterol molar ratio changed from 30 % to 35 %.

Abstracts

– Proton coupled electron transfer in photosynthesis –

P-300

Atomic structure of the bacteriochlorophyll c assembly in chlorosomes specialized for capturing weak light

A. Egawa[1], T. Fujiwara[1], T. Mizoguchi[2], Y. Kakitani[2], Y. Koyama[2], H. Akutsu[1]

[1]Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita 565-0871 Japan, [2]Faculty of Science and Technology, Kwansei Gakuin University, Gakuen, Sanda 669-1337, Japan

We have determined the tertiary structure of the bacteriochlorophyll *c* (BChl *c*) assembly in a huge light-harvesting organelle, the chlorosome of a green photosynthetic bacterium *Chlorobium limicola*, for the first time by solid-state nuclear magnetic resonance (NMR). Previous studies indicated that chlorosomes have a cylindrical architecture with a diameter of about 10 nm consisting of layered BChl molecules. Using ^{13}C dipolar spin-diffusion solid-state NMR of uniformly ^{13}C-labeled chlorosomes under magic-angle spinning, about 90 intermolecular C–C distances were obtained. Simultaneous assignment of distance correlations and structure optimization preceded by polarization-transfer matrix analysis was carried out. It has turned out that BChl *c* molecules form piggyback dimmer-based parallel layers, including inter-column hydrogen bonds. A molecular model of the cylinder in the chlorosome was built using this structure. The chlorosome structure provided new insights into light harvesting under low density photons: the high-density cylindrical arrangement of BChls *c* enables both efficient light absorption and excitation transfer to the reaction centers. The similar arrangements of Q_y transition dipoles for the assembly in chlorosomes and the B850 ring in LH2 suggest a common excitation transfer mechanism in the two systems.

P-301

Mathematical formulation of oxygen evolution in photosynthesis using a Langmuir equation

M. Fragata, V. Viruvuru

Univ. du Québec à Trois-Rivières, Dept. Chimie-Biologie, Canada

Electron transport through photosystem II (PSII), measured as oxygen evolution, was investigated in isolated PSII particles and photosynthetic membranes irradiated with white light of intensities (*I*) of 20 to about 4000 micromol of photons/m^2.s. In steady-state conditions, the evolution of oxygen varies with *I* according to the expression OE_{th}=OE_{th}(max)I/($L_{\frac{1}{2}}$ + I) (*eq 1*), where OE_{th} is the theoretical oxygen evolution, OE_{th}(max) is the maximum oxygen evolution, and $L_{\frac{1}{2}}$ is the light intensity giving OE_{th}(max)/2. The mathematical derivation of this relationship was performed using a Langmuir adsorption isotherm, and taking the photons interactions with the chlorophylls (Chl) in the PSII reaction center as heterogeneous reactions in which the light is represented as a stream of particles. In accordance with this approximation, the Chl molecules (P680) are taken as the adsorption surfaces (or heterogeneous catalysts), and the incident photons as the substrate. We demonstrated that *eq 1* (*Langmuir equation*) is a reliable interpretation of the photon-P680 interaction and the subsequent electron transfer from the excited state P680 (P680*) to the oxidized pheophytin (Phe), then from Phe- to the primary quinone Q_A. Interestingly, *eq 1* contains specific functional and structural information which is apparent in the definition of OE_{th}(max) as a measure of the maximal number of PSII reaction centers open for photochemistry, and $L_{\frac{1}{2}}$ as the equilibrium between the electron transfer from Phe- to Q_A and the formation of reduced Phe in the PSII reaction center by electrons in provenance from P680*. (*Supported by NSERC Canada*)

P-302

THz radiation from bacteriorhodopsin generated by light-induced electron and proton translocation

G. I. Groma[1], J. Hebling[2], I. Z. Kozma[3], G. Váró[1], J. Kuhl[4], E. Riedle[3]

[1]Institute of Biophysics, Biological Research Center, Szeged, Hungary, [2]Department of Experimental Physics, University of Pécs, Pécs, Hungary, [3]Chair for BioMolecular Optics, Ludwig-Maximilians-University, Munich, Germany, [4]Max Planck Institute for Solid State Research, Stuttgart, Germany

The primary charge separation processes following the excitation of the retinal chromophore and constructing the actual conversion of light energy into electrical one are hardly studied, due to the lack of conventional direct electric measurement techniques in the sub-ps domain. Here we introduce an alternative experimental approach, based on the familiar Hertz dipole radiation applied to the temporal and spatial range of the intramolecular charge translocation processes. Time-resolved THz emission from dried oriented purple membrane samples exposed to 100 fs laser pulses was detected by electrooptic sampling. According to our model calculations, the major component of the radiation can be attributed to an electron polarization correlating with the 500 fs life-time of the retinal excited state in native bR as well as with the prolonged one in its acid blue form. An additional phase of 3 ps decay and opposite sign was interpreted as the earliest step of the proton pump, presumably taking place in a H-bond near the retinal. Our results indicate that the energy conversion in bR is probably governed by harmonized ultrafast motion of both electrons and protons. This view corresponds to the extension of Mitchell's chemiosmotic theory into the range of femtosecond electrodynamics.

P-303

Temperature-induced changes in thylakoid membranes with different structural organization

P. I. Ivanova[1], A. G. Dobrikova[1], S. G. Taneva[2], E. L. Apostolova[1]

[1]Institute of Biophysics, Bulgarian Academy of Sciences, Acad.G.Bonchev Str., Bl. 21, [2]Unidad de Biofísica (CSIC/UPV-EHU) y Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad del País Vasco, Spain

The effect of heat treatment on chlorophyll fluorescence and photosynthetic oxygen evolution in pea thylakoid membranes with different organization of supramolecular complex of photosystem II (PSII) was investigated. Data show that heat-treatment of membranes leads to changes in the energy distribution between the two photosystems and inhibition of the oxygen evolution. Relationship between the amount and oligomerization state of the main light-harvesting antenna of photosystem II (LHCII) and the heat-induced changes in the energy distribution between the two photosystems and in the photosynthetic oxygen evolution was found. Lower content and degree of organization of LHCII in thylakoid membranes is related to higher susceptibility of the function of the photosynthetic apparatus to high-temperatures.

Acknowledgements: The work was supported by contract B-1512/05 with National Science Fund.

Abstracts

– Proton coupled electron transfer in photosynthesis –

P-304

Different responses of photosynthetic apparatus to action potential in high and low light conditions

N. A. Krupenina, A. A. Bulychev
Biophysics Department, Faculty of Biology, Lomonosov Moscow State University, Moscow, 119992, Russia

Chara corallina cells represent a convenient model to study plant cell excitability in relation to spatial patterns of H^+ fluxes and photosynthesis. Triggering of action potential (AP) was found to transiently suppress photosynthesis, disrupt H^+ fluxes across the plasmalemma in illuminated cells and give rise to non-photochemical quenching (NPQ). The sensitivity of NPQ to nigericin and a rapid release of AP-triggered NPQ in darkness indicate its relation to ΔpH at the thylakoid membrane. Evidence was obtained that the thylakoid ΔpH is subject to spatial pattern coordinated with the pH in the external medium. The sigmoid dependence of NPQ on photon flux density (PFD) shifted after cell excitation towards lower PFD. By comparing light intensity plots for NPQ, effective quantum yield of PSII electron flow (ΔF/Fm') and photochemical quenching qP, we found that the reduction in ΔF/Fm' with the increase in PFD involves different mechanisms at lower and higher PFD: the increase in thermal losses and the decrease in qP, respectively. In accordance with these variations, the AP-induced decrease in ΔF/Fm' was associated with a drop of Fm' (increase in NPQ) at low PFD and with an increase in actual fluorescence Ft (decline in qP) at high PFD. We examined the effect of Ca^{2+} ionophore A23187 on NPQ plots. The ionophore shifted NPQ plots similarly to the AP effect, consistent with a likely role of a rise in the cytosolic Ca^{2+} level in the AP-induced quenching. The results suggest that a rapid electric signal of plasmalemma might exert long-lived effects on photosynthesis and Chl fluorescence through ion flux-mediated pathways.

O-305

Proton-coupled electron transfer. The mechanistic engine that drives photosynthesis

D. G. Nocera
Department of Chemistry, Massachusetts Institute of Technology, U.S.A.

The transport of electrons and protons in Photosystem II (PSII) occur along pathways that are orthogonal to each other. The recent crystal structures of PSII support suggestions that as the Oxygen Evolving Complex (OEC) steps through its various S-states, substrate derived protons are shuttled to the lumen via a proton exit channel, the headwater of which appears to be the D61 residue. D61 is diametrically opposite to Y Z , which has long been known to be the electron relay between the reaction center and OEC. In this way, the PCET pathway for water oxidation at OEC is orthoganilised. Moreover, the oxidation of YZ occurs along an orthogonal PCET pathway. We have designed systems that capture orthogonal PCET and show that (i) proton motion can affect electron transport even when the electron and proton move along orthogonal coordinates and (ii) uncover the reason why PSII has designed orthogonal PCET pathways for substrate activation and radical transport.

O-306

Mechanisms of proton-coupled electron transfer from tyrosine and tryptophan in model complexes

M. Sjödin, T. Irebo, O. Johansson, L. Hammarström
The Ångström Laboratories, Uppsala University, Sweden

The redox reactions of radical proteins often involves bi-directional, proton-coupled electron transfer (PCET) from or to amino acid residues, in which the proton and electron are transferred in different directions. We previously demonstrated the first bi-directional PCET in a synthetic model complex comprising a tyrosine residue covalently linked to a Ru^{II}-polypyridine photosensitizer.(1) The PCET was a *concerted* process, displaying a previously unreported type of pH-dependent rate. Detailed studies of PCET in new Ru-Tyr and Ru-Trp complexes showed that we could deliberately switch the mechanism between concerted and step-wise PCET.(2) Within a new mechanistic model, we obtained quantitative data for the parameters that govern the competition between these mechanisms. A key parameter is the reorganization energy, which we showed is high for a concerted reaction.

Our results and a detailed comparison with Tyr_Z oxidation in Photosystem II allowed us to draw new conclusions about the mechanism of the natural system. Our new results from hydrogen-bonded systems suggest that the hydrogen bond reduces the reorganization energy for a concerted reaction, which increases the PCET rate.(3) We can also present new experimental data for a complete "rate ladder" which we previously predicted, on which the different "steps" depend on the protonation or hydrogen bonding situation of the tyrosine.(4)

1 Sjödin, et al, *J. Am. Chem. Soc.* **2000**, *122*, 3932-3936.
2 Sjödin, et al, *J. Am. Chem. Soc.* **2005**, *127*, 3855-3863.
3 Sjödin,et al, *J. Am. Chem. Soc.* **2006**, *128*, 13076.
4 Irebo, Johansson, Hammarström, *in progress.*

O-307

Design features of long distance proton transport systems in biology

C. A. Wraight
Department of Biochemistry, and Center for Biophysics & Computational Biology, University of Illinois at Urbana-Champaign, USA

Long distance proton transport is a critical function in biology, especially in bienergetics, and is also of great importance in low temperature fuel cell technology, based on polyelectrolyte membranes. However, the issues of biological charge (ion) transport are very different from those of synthetic devices. With physiological concentrations of K^+ and Na^+ in the 0.1 M range, and H^+ only $\approx 10^{-7}$ M, high specificity and high throughput at low concentration are the major challenges. At the same time, the underlying biological principles must provide evolutionary robustness rather than durability. How biology addresses these issues indicates the key role of internal water for high turnover, coupled with specific mechanisms to achieve enhanced H^+ availability and very high selectivity. Examples are evident among ion channels and bioenergetic H^+ pumps and in non-vectorial H^+ uptake. In non-vectorial proton-coupled redox chemistry, the involvement of metastable intermediates in multi-electron events (charge accumulation) may require sequestration of redox centers, necessitating long distance proton transfer pathways, with similar design requirements.

References

Wraight, C. A. (2006) Chance and Design – Proton transfer in water, channels and bioenergetic proteins. *Biochim. Biophys. Acta* 1757, 886-912.
Takahashi, E. and C. A. Wraight (2006) Small weak acids reactivate proton transfer in reaction centers from *Rhodobacter sphaeroides* mutated at Asp^{L210} and Asp^{M17}. *J. Biol. Chem.* 281, 4413-4422.

Abstracts

– Proton coupled electron transfer in photosynthesis –

O-308

Exposing the complex III Qo semiquinone radical

H. Zhang[1], A. Osyczka[2], C. C. Moser[1], P. L. Dutton[1]
[1]Department of Biochemistry & Biophysics, University of Pennsylvania, Philadelphia, PA, U.S.A., [2]Department of Biophysics, Jagiellonian University, Krakow, Poland

There are a growing number of examples that support the importance of high tunnelling frequencies in redox catalysis. Current work on hydroquinone/quinone (QH_2/Q) mediated energy conversion in Complex III in the respiratory chain illustrates this nicely. The Qo site of Complex III is well known to reversible catalyse two-electron, two-proton QH_2/Q oxidation/reduction coupled to transmembrane charge-separation across respiratory and photosynthetic membranes. However the molecular details of the reaction remain uncertain. For 30 years, a semiquinone SQ radical has been proposed as pivotal not only as a radical intermediate in productive energy-conversion but also as a source of superoxide that signals cellular protection and/or initiates regulated cell death (apoptosis). Recently we have exposed a SQ state associated with the Qo site, observed as an EPR radical[1]. We used a genetic knockout of a peripheral heme cofactor (b_H) of Complex III to limit the capacity of the low potential b-chain to accept electrons. The radical is highly reactive with estimates of the QH_2/SQ redox level at about +0.41V and SQ/Q redox level at –0.47V (SHE at pH 9). Tunnelling calculations suggest that the uphill barrier to the intermediate SQ state is close to the limit possible to support millisecond catalysis perhaps to minimise the level and lifetime of a highly reactive radical.

1 Zhang, H., Osyczka, A., Dutton, P.L., and Moser, C.C., *Biochem. Biophysics. Acta.* In press, April 2006.

Abstracts

– DNA/lipid complexes and drug delivery –

P-309

Cubic membranes: a structure-based design for a new DNA vector?

Z. Almsherqi[1], S. Hyde[2], R. Malarmathy[1], Y. Deng[1]

[1]Dpt. of Physiology, NUS, Singapore, [2]Dpt. of Applied Mathematics, ANU, Singapore

A significant obstacle of gene therapy is the potential toxicity and inefficiency of current delivery vehicles. It is of interest to explore an alternate delivery media with the features of efficient DNA packaging, release and nucleus targeting in addition to the low toxicity. Cubic phase is a promising structure for drug delivery due to its amphiphilic nature, ability to incorporate and to control release of drugs as well as the biodegradability. Because of its structural resemblance to cubic phases, cubic membrane observed in numerous cell types was proposed to play a role in macromolecule transportation. Here we investigated the possibility that cubic membranes are able to uptake short segments of DNA. Mitochondria with cubic membrane organization were isolated from 10-d starved amoeba *Chaos*. Using flourscence microscopy we demonstrated that MitoTracker Red-labeled mitochondria with cubic membrane structure are able to uptake and retain green fluorescent-tagged oligonucleotides (ODN). TEM studies further demonstrated a significant interaction between ODN and cubic membrane, which is able to retard electrophoretic mobility of ODN component in the gel matrix. Finally, we showed that targeted cells could uptake fragmented ODN-cubic mitochondria complexes efficiently. Our results show that mitochondria containing ordered cubic sponges containing hyperbolically curved membranes readily adsorb DNA, with significant molecular uptake. Although at this stage it is difficult to offer a definitive explanation for the mechanism of DNA uptake of cubic membrane structure, however, it may afford a new transfection vector for at least short ODNs such as siRNA.

P-310

Influence of external noises on binding of ligands with DNA

A. Arakelyan, Z. Navoyan, V. B. Arakelyan

Yerevan State University, Yerevan, 0049, Armenia

The influence of external Langevin and multiplicative noises on binding of ligands to DNA is investigated. It is shown that Langevin noise does not influence on the average quantity of DNA bound ligands. Non-stationary values of variance, correlation function and spectral density of the number of DNA adsorbed ligands in presence of Langevin external noise were calculated. It is shown that the variance linearly grows with the growth of intensity of external noise. Spectral density of the number of DNA bound ligands has a Lorentzian form. It is also shown that through the correlation function or spectral density analysis it is possible to separate "fast" and "slow" adsorption of ligands on DNA. The influence of external multiplicative noise on binding of ligands to DNA strongly differs from the influence of Langevin noise. It is shown that due to the influence of multiplicative noise the average number of DNA bound ligands becomes dependent on the intensity of the external noise. At certain proportion of noise intensity and the ligands' quantity in the solution, adsorption of ligands on DNA does not take place. In presence of multiplicative noise, the variance of the quantity of DNA bound ligands grows with the growth of intensity of external noise.

P-311

Interaction of DNA with mixed lipid monolayers studied by neutron scattering

A. P. Dabkowska[1], M. J. Lawrence[1], A. V. Hughes[2]

[1]Pharmaceutical Sciences Division, King´s College London, UK, [2]Rutherford Appleton Laboratories, Didcot, Oxfordshire, UK.

The interaction between zwitterionic phospholipids and negatively charged DNA, mediated by a divalent cation, is an exciting possibility for the development of safe gene vectors. The present study has investigated the nature of this interaction by determining the structure of a mixed phospholipid film at the air-water interface, in the presence of Ca^{2+} and DNA using Langmuir isotherms, neutron reflectivity (NR) in combination with contrast variation and Brewster angle microscopy (BAM). The lipid films studies, which consisted of distearoylphosphatidylcholine (DSPC) and a neutral "helper lipid", either cholesterol or phospatidyethanolamine, were examined at 4 surface pressures (π) of 10, 20, 30 and 40 mN/m. For the NR experiments, 3 contrasts, namely deuterated DSPC, hydrogenous helper lipid in D_2O and air contrast matched water (acmw) and hydrogenous DSPC and helper lipid in D_2O were examined. Data was analysed using Motofit software. Although π-A isotherms suggested that, in the presence of Ca^{2+} DNA interacts with lipid film monolayers only at low π, NR showed that the interaction occurs regardless of π and helper lipid used. Using NR a second layer, containing 20 vol% DNA with the thickness of double stranded DNA, was present below the lipid film in the presence of Ca^{2+}. In the absence of Ca^{2+}, this layer of DNA was absent indicating that DNA does not interact with zwitterionic monolayers without divalent ions. BAM studies confirmed this interaction. Results of these studies will aid in the rational design of gene vectors.

O-312

Can lipid domains control drugs and DNA uptake in cells submitted to electric fields?

J.-M. Escoffre[1], F. Camps[1], M. Hubert[1], C. Favard[2], J. Teissie[1], D. Dean[3], M.-P. Rols[1]

[1]Institut Pharmacologie Biologie Structurale-CNRS UMR 5089 Toulouse France, [2]Institut Fresnel UMR 6133 Marseille France, [3]Laboratoire Physique Théorique UMR 5152 Toulouse France

Electropermeabilization can be used to transfer molecules into cells. Although this approach shows promise, little is known on the basic processes supporting the transfer (1). We visualized at the single-cell level the early events of membrane permeabilization and DNA transfer in cells by electric field. A fast exchange of small hydrophilic molecules takes place across the membrane. In the case of DNA, a complex process is present involving a key step of association of DNA with the membrane (2).

The purpose of our present work is to better understand the molecular state of the electropermeabilized cell membrane. We carry out a detailed study of how the application of an electric field modifies the transport properties of artificial (GUV) and cell membranes. The transverse transport properties which involve the crossing of the membrane are the most relevant for gene therapy and chemotherapy, are studied experimentally using an ultra-fast imaging set up. The influence of electric fields on lateral transport properties are studied by FRAP measurements. For large molecules such as DNA, membrane permeabilization is characterized as a two stage process, the first being the interaction of the plasmid with membrane domains where DNA is trapped and the second being the translocation of the plasmid through the membrane.

1-Favard C. et al. Curr Gene Ther. (2007) 7:67-77.

2-Golzio M., Teissié J. and Rols MP. PNAS *USA*. (2002) 99: 1292-7

Abstracts

– DNA/lipid complexes and drug delivery –

P-313

Oxidation of acetaminophen by methemoglobin in the presence of hydrogen peroxide

M. I. González[1], F. Garcia-Carmona[2], R. Varón[1], M. Fuentes[1], E. Arribas[1], E. Valero[1]
[1]Escuela Politecnica Superior. universidad de Castilla-La Mancha, Albacete, Spain., [2]Facultad de Biología. Universidad de Murcia, Murcia, Spain.

It has been known for a long time that hemoglobin performs peroxidative reactions in presence of H_2O_2, analogous to conventional peroxidases. The oxidation of acetaminophen by methemoglobin in the presence of H_2O_2 was kinetically studied in the present comunication. Contrary to that expected, the protein exhibits non-Michaelian kinetics both against acetaminophen and H_2O_2 under steady-state conditions. These data were therefore compared to those obtained with myoglobin under similar experimental conditions, and the same results were observed. This led to us to propose a new mechanism, including the possible formation of a new intermediate of hemoglobin, compound III, by reaction of compound II with H_2O_2, able to react with a new molecule of acetaminophen.
Experiments until either acetaminophen or H_2O_2 were depleted in the reaction medium have also been perfomed to stablish the stoichiometry of the pathway between the drug, H_2O_2 and hemoglobin. In addition, hydroxyl radical scavengers such as mannitol, dimetyl sulfoxide or ethanol, scarcely inhibited acetaminophen oxidation by metHb with H_2O_2, indicating that the hydroxyl radical is not involved in the reaction.
Acknowledgements: PAI-05-036 (JCCM, Spain) and BQU2002-01960 (MEC).

O-314

Cell-penetrating peptides can translocate without pore formation. A biophysical study with pep-1

S. T. Henriques[1], F. Homblé[2], L. A. Bagatolli[3], M. A. Castanho[1]
[1]CQB, FCUL, Lisboa, Portugal, [2]SFBF, ULB, Brussels, Belgium, [3]MEMPHYS, SDU, Odense, Denmark

The cell-penetrating peptide pep-1 is able to introduce proteins into different cell lines, maintaining their biological activity; however the uptake mechanism is unknown. Our aim is to understand pep-1 interaction with biological membranes and to elucidate the peptide cellular uptake. Pep-1 translocation *in vitro* and *in vivo* only occurs in the presence of transmembrane potential without evidence for an endosomal-dependent mechanism, which suggests that the underlying translocation mechanism is physically-mediated. Formation of a transmembrane pore was proposed by others and this issue has remained controversial. Secondary structure of pep-1 in the absence/presence of lipidic bilayers was determined by CD and ATR-FTIR spectroscopies. The occurrence of pore formation was evaluated through electrophysiological measurements with planar lipid membranes and by confocal microscopy using giant unilamellar vesicles. Upon interaction with membrane, hydrophobic domain has a tendency to form alpha-helix and has a tilt angle when inserted in membrane. Despite this tendency for alpha-helix conformation in the presence of lipidic bilayers, there was no evidence for membrane pores in the presence of pep-1. Furthermore, alterations in membrane permeability only occurred for high peptide/lipid ratios, which induced the complete membrane disintegration. Such observations indicate that electrostatic interactions are of first importance in pep-1-membrane interactions and show that pores are not formed. A carpet-model mechanism, mediated by peptide affinity for membranes, occurs but only in extreme conditions.

P-315

Dynamic light scattering and dielectric spectroscopy of DNA and liposome samples in dilute solutions

S. K. Kundu, S. G. Choe, R. Kita, N. Shinyashiki, S. Yagihara
Department of Physics, School of Science, Tokai University, 1117 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan

For designing liposome-based drug carrier systems, a reliable and reproducible analysis of their size and size distribution is of paramount importance. Liposome size influences the nanocarrier's in-vitro characteristics such as drug loading capacity, aggregation and sedimentation. Broadband dielectric spectroscopy has been used to investigate the dynamical structure of water surrounding DNA and liposome in aqueous solutions. For both DNA and Liposome, we have observed two relaxation processes in the frequency range between 1MHz to 30GHz. For both cases, the high frequency process appearing about 20 GHz is due to the free water surrounding DNA and liposome molecules. The second process appearing in the MHz region is due to the dynamic behaviors of ions, which is also related to the molecular motion of the lipid. For DNA, the second process appearing around 50 MHz region is due to bound water and it is also related to the molecular motion of the DNA with ions. Dynamic light scattering experiment has been used to investigate the size, size distribution and diffusion of DNA, liposome and their mixtures (with a weight ratio 1:1) in aqueous solutions. Dynamic light scattering of DNA-liposome mixture shows two classes of particle, their mean hydrodynamic radius being 65-75 nm and 950-970 nm. Observation of the large particles in the solution indicates that the lipoplex (DNA+Liposome) are formed by addition of DNA solution to the liposome suspension. Complex morphology occurring in lipid-DNA lipoplex formation could provide insight for the development of efficient artificial delivery systems in gene therapy.

P-316

Interaction between a non ionic lipid and DNA_the lipopolythiourea way

J. Leblond[3], J. Herscovici[3], C. Largeau[4], J. Seguin[2], M. F. Bureau[2], D. Scherman[1], N. Mignet[2]
[1]Inserm, U640, Paris, F-75006 France, [2]CNRS, UMR8151, Paris, F-75006 France, [3]Ecole Nationale Supérieure de Chimie de Paris, Paris, F-75005 France, [4]Université Paris Descartes, Faculté de Pharmacie, Paris, F-75006 France

Lipopolythiourea (LPT) are original non cationic systems representing an alternative to cationic lipids. The non cationic character of lipopolythiourea was demonstrated by titration and zeta potential measurements. Interaction of lipopolythiourea with DNA was investigated. Gel electrophoresis, circular dichroism and ^{1}H NMR experiments revealed a strong interaction between lipopolythiourea and DNA, occurring at 1 equivalent of thiourea function by DNA phosphate group, and leading to a condensed plasmid state. Moreover, in vitro evaluation of the complexes formed indicates the ability of these systems to efficiently transfect cells. Upon local injections in vivo, LPT-DNA complexes did not allow improving muscle transfection as compared to electrotransfered naked DNA. In opposite, intratumoral injection of the complexes led to improved tumor transfection as compared to naked DNA. Finally, when injected intravenously, their blood retention was improved as compared to conventional liposomes.

Abstracts

– DNA/lipid complexes and drug delivery –

P-317

Cell membrane alterations by weak alternating electric field at low frequencies

B. P. Nikolova, I. C. Tsoneva
Institute of Biophysics, Bulg. Acad. Sci., Sofia, Bulgaria

We have demonstrated that some malignant and normal hematopoietic cells (HL-60, SKW3 and lymphocytes) and E. coli could be electroporated by the weak alternating electric field (effective field strength of 100 V.cm^{-1}) and low frequencies (0.1-25 Hz), the time of the treatment - 10s. According to Laplace equation, such low intensity is not able to porate single biological membrane. Furthermore, E. coli cells could be transformed at the above electrical parameters. Transfection frequencies depend on: the geometry of the used chambers, the concentration of particles, and the viscosity of the medium. The number of transformed cells increases two times when some special chambers for the amplification of oscillating field are used. In this case the alternating electric field is generated by applying voltage across two platinum wires and between them are incorporated the strips of very thin gold film to generate strong electric fields with steep gradients. The amplification of field strength at the edges of the gold-film strips is given by Asbury, L. C., Engh, G. A. [1] and Ajdary, A. and Prost, J. [2]. The maximum of the electroporation is at 1 Hz.
In the work, “the unusual behavior” of cells and the amplification of the alternating electric field at very low frequencies are discussed.
The future goal of the work is the application of weak ac fields and low frequencies of 1-10Hz at the low pain electrochemotherapy application.
Acknowledgments: We thank for the financial support by BNF-K1303

P-318

Finding AT-DNA – kinetic recognition of long adenine-thymine stretches by metal-ligand complexes

P. Nordell[1], F. Westerlund[2], L. M. Wilhelmsson[1], B. Nordén[1], P. Lincoln[1]
[1]Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, [2]Nano Science Center, University of Copenhagen, Copenhagen, Denmark

Threading of bulky binuclear ruthenium(II) complexes in or out of the base stack in DNA is an extremely slow process that requires a considerable conformational change of the double helix. The rate of intercalation is highly sensitive to the structure and stereochemistry of the metal-ligand coordination and, in particular, to the base sequence around the intercalation site: 2500 times faster with poly(dAdT)$_2$ compared to mixed-sequence DNA for the most selective complex. Studies with oligonucleotides having a central AT-tract of varying length demonstrate that at least one complete helix turn of AT basepairs is necessary for efficient threading, indicating that the transition state involves a stretch of DNA considerably larger than the dimensions of the complex itself. Our results demonstrate that this "kinetic recognition", in which the selection mechanisms go beyond the short-range interactions of a simple lock and key model, can provide a highly sequence-selective DNA interaction.
Key references:
[1] L. M. Wilhelmsson, F. Westerlund, P. Lincoln, B. Nordén, *J. Am. Chem. Soc.* **2002**, *124*, 12092.
[2] P. Nordell, P. Lincoln, *J. Am. Chem. Soc.* **2005**, *127*, 9670.
[3] P. Nordell, F. Westerlund, L. M. Wilhelmsson, B. Nordén, P. Lincoln, Kinetic Recognition of AT DNA by Ruthenium Complexes, *Angew. Chem., Int. Ed.* **2007**, *46*, 2203.

P-319

In vitro study of mechanisms involved in gene electrotransfer using high- and low-voltage pulses

M. Pavlin, M. Kanduser, D. Miklavcic
University of Ljubljana, Faculty of Electrical Engineering, Slovenia

Electroporation is a biotechnology technique that among other allows transfer of DNA into biological cells, known as gene electrotransfer. High-voltage pulses induce structural changes - pores in the cell membrane, which becomes transiently permeable for ions, molecules and macromolecules, that otherwise cannot permeate through the membrane. The electrogene transfer is already an established method for gene transfer in vitro and in vivo. Currently, the majority of research is focused on improving in vivo transfection efficiency and in clinical trials even though the exact mechanisms involved in electrotransfer of DNA across the cell membrane are not yet established. Therefore in our present study we investigate mechanisms of gene electrotransfer in vitro by using combination of high-voltage (HV) and low (LV) voltage pulses. This specific combination showed improved transfection efficiency in vivo compared to only HV pulses. It was suggested that HV electric pulses destabilize the cell membrane and enable insertion of DNA into the cell membrane, whereas LV pulses provide electrophoretic force which drives charged DNA molecule into the cell. Transfection efficiency was determined using fluorescent microscopy using GFP coding plasmid on CHO cells. We show that the effect of LV pulse can not be explained only as an electrophoretic force which drives DNA molecules and the role of HV pulse as only increasing permeability of the cell membrane. We analyze different possible mechanisms which are involved and suggest that discrepancies between results obtained in vivo and in vitro could be explained with the effect of LV pulse on mobility of DNA in in vivo conditions.

O-320

Stochasticity in artificial gene transfer: single cell gene expression experiments and modelling

J. Raedler
Ludwig-Maximilians-Universität, Munchen, Germany

The transfer of foreign DNA into eucaryotic cells requires artificial vectors as physical carriers. Such nanoparticles are subject to general transport phenomena in soft matter, such as diffusion in polymer networks, particle membrane interaction and directed transport towards the nucleus using microtubular trackage. Using enhanced green fluorescence protein (EGFP) as a reporter, gene expression in a large number of individual cells in culture was monitored by semi-automated time-lapse fluorescence microscopy. The time courses are described by a linear gene expression model that includes the maturation kinetic of EGFP into its fluorescent state. The analysis captures the stochasticity in the gene expression kinetics within a cell culture populations. The distributions of expression onset times, expression rates at half maximum intensity, and steady state EGFP fluorescence intensities were determined for two synthetic gene delivery systems, linear polyethyleneimine (PEI) and lipofectamine. It is proposed that the variance in single cell GFP expression reflects a Poisson like distribution of nuclear entry events. Hence noise in gene expression is dominated by the intrinsically stochastic nature of the delivery process rather than to depend on cell-to-cell variability.

Abstracts

– DNA/lipid complexes and drug delivery –

O-321

Single molecule imaging and force spectroscopy of nucleic acid-protein complexes

C. J. Roberts, S. Allen, P. M. Williams
School of Pharmacy, the university of Nottingham, U.K.

The ability to visualize and manipulate individual biomolecules in environments relevant to their function and in particular to challenge such systems during these measurements is providing new insights into many biomolecular systems. We will present image and single molecule force spectroscopy data highlighting the role such information can play in understanding polymeric-nucleic acid interactions in the design of gene delivery systems, in small molecule-nucleic acid interactions and in revealing novel functional roles for replisomal bacterial proteins in nucleic acid replication and macroscopic re-modelling.

P-322

Nucleic acid interaction with zwitterionic and anionic model lipid membranes

A. Zettergren[1], N. Kristen[1], M.-L. Örberg[1], F. Höök[2], T. Nylander[1], E. Sparr[1]
[1]Physical Chemistry 1, Lund University, Sweden, [2]Solid state physics, Lund University, Sweden

In the living system, RNA is present in an environment that contains many substances, which can influence its structure and function. RNA occurs more often than DNA in the single-stranded form, where the apolar parts of the bases are obvious targets for interaction with hydrophobic species in the environment, e.g. lipids in the cell membrane and in the cell nuclei. The objective of this work is to investigate how RNA (ribonucleic acid) interacts with lipid membranes that have a composition similar to the one found in plasma membranes and in the cell nucleus. We have investigated interactions between tRNA (transfer RNA), and vesicles composed of zwitterionic PC (phosphatidylcholine) and anionic PS (phosphatidylserine) with DSC (differential scanning calorimetry) and QCM-D (quartz crystal microbalance with dissipation). QCM studies indicate that RNA associates with the lipid membrane and that there is a gradual uptake of RNA to the membrane surface over time. DSC measurements show an increase in lipid chain melting temperature in the presence of tRNA. This implies that the L_β gel phase is stabilized over the L_α liquid crystalline phase and it indicates that the association likely takes place in the interfacial region of the bilayer. The results are compared to studies on the interaction between phospholipids bilayers and double and single stranded DNA.

Abstracts

– Live cell imaging –

P-323

Applications of quantum dots for single molecule imaging in cells and substrate-supported membranes

E. Arnspang Christensen, H. Matras, C. B. Lagerholm
MEMPHYS, University of Southern Denmark, Odense, Denmark

Quantum dots have several advantages compared to other probes for single molecule imaging. These include enhanced brightness and photostability as well as in some cases smaller size. Perhaps the major advantage of quantum dots for single molecule imaging is the possibility of simultaneous imaging of multiple species at fast repetition rates over long periods of time. With this in mind, we have begun assembling a microscopy system eventually capable of imaging multiple colors of single quantum dots at high repetition rates over long periods of time in cells and substrate-supported planar membranes. With our current system, which consists of an Olympus IX81 microscope equipped with a 100 W Hg arc lamp for excitation and an electron-multiplied CCD (Andor DV887-ECS) for detection, we can in some cases image single quantum dots with 100 μs signal integration or at rates up to about 250 Hz. These results are however very dependent on the particular emission color characteristics of the quantum dots, as we find that certain quantum dot colors are dimmer and/or primarily in a non-fluorescent state. We will present data on the intensity and on/off characteristics of a variety of quantum dots. We will also give examples of single molecule imaging with quantum dots for tracking membrane proteins and biotin lipids in cells and biotin lipids in planar substrate-supported membranes.

P-324

Size of the fusion pore formed during Weibel-Palade body kiss-fusion events

V. Babich, L. Knipe, M. J. Hannah, T. Carter
MRC, National Institute for Medical Research, London, UK

Weibel-Palade bodies (WPB) are endothelial cell specific secretory organelles. They contain von Willebrand factor (VWF), the VWF propolypeptide (proregion), P-selectin and, under some conditions, a range of small peptide chemokines and cytokines. Expression of proregion fused to green fluorescent protein (EGFP) fluorescently labels fusion competent WPB in living human umbilical vein endothelial cells. Kiss-fusion events seen during histamine (100μM) or ionomycin (1μM) stimulation were characterised by an initial increase in intra-WPB EGFP-fluorescence due to the rise in intra-WPB pH following formation of a fusion pore, an abrupt change in WPB morphology without loss of proregion and intra-WPB accumulation of the extracellular fluid phase marker Alexa-647. To estimate the size of the fusion pore formed during these kiss-fusion events simultaneous triple colour confocal microscopy was used to monitor fusion pore formation, using intra-WPB EGFP and extracellular Alexa-647, and the intra-WPB accumulation of tetramethylrhodamine (TMR) conjugated to dextrans of different sizes (3-2000KDa). TMR-dextrans of 3 to 40KDa accumulated within the WPB structures following fusion pore formation; however, 70 and 2000 KDa TMR-dextrans were excluded. 40 and 70KDa dextrans have Stokes' radii of $\sim$9 and 12 nm respectively. Although proregion ($\sim$100KDa) does not exit through the pore formed during WPB kiss-fusion events, the size of the structure indicated by permeation of TMR-dextrans indicates that ions and small molecules, that can be co-packaged with VWF in WPB, may escape providing a potential mechanism for differential granule content release.

P-325

Dispersal of Weibel-Palade body cargo proteins following exocytosis is sensitive to external pH

V. Babich[1], L. Knipe[1], M. J. Hannah[1], J. Dempster[2], T. Carter[1]
[1]MRC, National Institute for Medical Research, London, UK, [2]University of Strathclyde, Glasgow, UK

Weibel-Palade bodies (WPB) are endothelial cell secretory organelles containing von Willebrand factor (VWF) the VWF-propolypeptide (Proregion), P-selectin, CD63, and under inflammatory conditions small peptide cytokines (e.g. IL-8) and chemokines (e.g. Eotaxin-3). Inflammation and ischemia are associated with tissue acidification, but little is known about the consequences of extracellular acidification for release and dispersal of secreted WPB cargo molecules. The effect of extracellular pH on the dispersal of VWF, Proregion, IL-8, Eoxtain-3, P-selectin and CD63, following exocytosis of individual WPB in living HUVEC was examined. HUVECs co-expressing EGFP and mRFP chimeras of WPB cargo molecules were imaged. WPB exocytosis was evoked by histamine (100μM) or ionomycin (1μM) in physiological saline solution buffered to pH7.4, 7.0 or 6.5. At pH7.4, exocytosis of WPBs containing fluorescent VWF and Proregion, was characterised by morphological changes, adhesion of VWF to the cell surface and rapid dispersal of Proregion into solution with a half time ($t_{1/2}$) of 3.5$\pm$0.6s. At pH7.0 Proregion dispersal was similar, however at pH 6.5, dispersal was significantly slowed, $t_{1/2}$=33.0$\pm$4.0s. In many cases the morphological changes associated with fusion were also less pronounced. At pH 7.4 IL-8, Eotaxin-3, CD63 and P-selectin dispersed from the site of WPB fusion with time-courses similar to that of Proregion. At pH6.5, dispersal of IL-8, Eotaxin-3 and CD63 was unaltered; however, P-selectin dispersal was significantly slowed.

O-326

Studying inter-cellular calcium wave propagation in pancreatic islets

R. K. Benninger[1], J. V. Rocheleau[1], M. Zhang[2], S. J. Le Marchand[1], S. W. Head[1], L. S. Satin[2], D. W. Piston[1]
[1]Vanderbilt University, Nashville TN, U.S.A., [2]Virginia Commonwealth University, Richmond, VA, U.S.A.

Islets of Langerhans are integral to maintaining blood glucose through secretion of hormones such as insulin. Beta cells respond to elevated blood glucose by a cascade of metabolic and electrical signaling to elevate intra-cellular free calcium levels and secrete insulin. The islet is organized such that the dynamic range of insulin response to glucose is many fold increased over the equivalent mass of isolated beta cells. Additionally intra-cellular calcium levels and insulin release are in a coordinated pulsatile manner with a sharper dose response to glucose than the heterogeneous responses observed in isolated beta cells.

At elevated glucose levels waves of intra-cellular calcium are observed to propagate across the islet, similar to that seen in, for example, the heart, or smooth muscle tissue. We have studied these waves in the islet using high-speed calcium imaging. A mean wave velocity of 75μm/s indicates that the waves are too fast to result from diffusion mediated paracrine communication. Application of gap junction blockers 18-α-glycyrrhetinic acid or mefloquine results in a slowing of the calcium wave, suggesting that the waves result from gap junction medicated electrical communication. This calcium wave slowing can be well described by a percolating model of conduction as confirmed by electrophysiological recordings. This therefore gives a reliable assay for islet gap junction coupling which will be important for further studying the precise role of gap junction mediated electrical coupling in islet function.

Abstracts

– Live cell imaging –

P-327

Endothelial fenestration characterized by AFM

C. Bodor[1], J. Nagy[2], A. Masszi[1], A. Jenei[2], S. Mirzahosseini[1], L. Mátyus[2], L. Rosivall[1]

[1]Institute of Pathophysiology, Hungarian Academy of Sciences and Semmelweis University Pediatric and Nephrology Research Group, Budapest, Hungary, [2]Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

The juxtaglomerular (JG) portion of afferent arteriole (AA) facing the renin expressing granular cells is fenestrated. To elucidate the intracellular mechanisms responsible for endothelial fenestration human umbilical vein endothelial cells were isolated and cultured and treated with recombinant VEGF or with its solvent. After fixing with ethanol, cell surface was screened by atomic force microscopy (AFM).

Endothelial permeability increased by more than 2-fold subsequently to VEGF treatment. This effect was detectable as early as after 15 minutes. In control cultures, only a few fenestrae could be visualized by AFM. However, after 15 min of VEGF treatment we detected increased fenestration which further increased after 48 hours of VEGF treatment. The total area of fenestrae also increased in VEGF treated cultures. The average size of these pores was 120-160 nm. VEGF induced rapid and sustained activation of p38 detectable even after 48 hours. Preincubation with SB203580, an inhibitor of p38 reduced VEGF-induced permeability measured by dextran diffusion. SB203580 significantly inhibited formation of fenestrae of VEGF treated cells.

In our experiments AFM is proved to be a useful technique for the characterization of endothelial fenestration, to gain high-resolution images of the fenestrae.

P-328

Riffles of the cell dynamics as exposed by wavelet analysis in the light of interference microscopy

A. Brazhe[1], N. A. Brazhe[1], A. Yusipovich[1], O. Sosnovtseva[3], A. Pavlov[2], G. V. Maksimov[1], E. Mosekilde[3]

[1]Biological fac., MSU, Russia, [2]Physics dep., SSU, Russia, [3]Physics dep., DTU, Denmark

Modern science of the cell virtually can't be imagined without advanced imaging techniques. However, most of the cell imaging techniques are more or less invasive, in the sense that they imply the use of dyes or drying or mechanical coercion. This can affect the normal cell function in an undesired way and one thus needs a convenient noninvasive technique. The use of intrinsic optical properties like local refractive index (RI) or light scattering as a noninvasive probe has been considered. Laser interference microscopy (LIM) is a modern and unique cell imaging technique which allows to see real-time changes in the local RI of a cell. In this work we present the results of the application of LIM and wavelet-analysis for cell visualization and studies of cell dynamics for several cell types. The interference imaging of red blood cells reveals reorganization of the cytoskeleton and inhomogeneous distribution of haemoglobin in the case of disease. The intracellular compartmentalization and submembrane structures are also clearly seen in isolated neurons and mast cells. The temporal variations of the local RI were also studied. We learned that low frequency variations (0.5-6 Hz) result from plasma membrane processes and higher frequency variations (15-25 Hz) result from vesicle movements and cytoskeleton reorganization. The set of detectable rhythms in the RI variations and their modulation patterns revealed by double wavelet analysis were shown to depend on the cell type and to be affected by chemical agents.

P-329

Mechanisms of nitric oxide influence on the membrane structures of the cell

N. A. Brazhe, G. V. Maksimov

Biophysics department, Moscow State University, Russia

Change of membranes properties is one of the main principles of the modulation of the nerve cell function. Nitric oxide (NO), being a hydrophobic molecule, easily penetrates into the cellular membranes and compartments. In the present work we show that NO influences various neuronal and axonal membrane structures and thus causes different time-scale changes of nerve cell properties. Using patch-clamp, extracellular electrodes, conventional and confocal fluorescent microscopies we studied effect of NO on the ion channel activity, cellular excitability, amount of bound Ca^{2+} and mitochondria function of neurons and nerve fibres. Our preparations were neurons and nerve fibres of the medical leech. As NO donors we used spermine/NO and sodium nitroprusside. We have shown, that exogenous NO firstly activates voltage-dependent K-channels, decreases nerves excitability and then evokes desorption of Ca^{2+} from the plasma membrane ($Ca^{2+}{}_{mb}$). Change of $Ca^{2+}{}_{mb}$ amount was depended on the K-channels activity and in its turn altered membrane surface charge. Besides, NO brought to the decrease in the plasma membrane fluidity. In the cytoplasm NO caused the decrease in the FAD^{+}/FADH ratio relating to the succinate-dehydrogenase activity, and long-lasting depolarization of mitochondria bringing them to release Ca^{2+} from the matrix. For the first time using interference microscopy we showed that NO influences neurons' refractive index due to the cooperative processes evoked by NO in the plasma membrane and cytoplasm. We suggest that prolonged changes of membranes and organelles properties may be among mechanisms that provide signal transduction.

P-330

Couped two-photon and second harmonic generation non-descanned microscopy for In-vivo imaging

M. Caccia[1], L. Sironi[1], G. Chirico[1], I. Zanoni[2], F. Granucci[2]

[1]Dipartimento di Fisica G. Occhialini, Università di Milano Bicocca, Milano 20125, Italy, [2]Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milano 20125, Italy

Effective applications of non-linear microscopy to in-vivo studies require the optimization of the collection efficiency. We report the characterization of a home-made non-descanned detection unit for two-photon imaging of biological tissues in-vivo and we describe the application of the system to Second Harmonic Anisotropy, (SHA), a technique that enables to study the degree of organization of the samples at molecular level and to recognize pathological situations.

The setup is coupled to a commercial scanning confocal microscope, and provides three high efficiency collection channels (two-photon and second harmonic scattering), thereby enabling to observe cellular dynamics within the tissue at low excitation intensity for tens of minutes to hours. The increase in the signal to noise ratio of the non-descanned imaging unit with respect to the confocal descanned one, ranges from $\cong$ 4-16, depending on the emission wavelength. This result is not always paralleled by an increase in the absolute signal on the non-descanned channels.

SHA is used to investigate the organization of collagen fibrils in poplyteal mouse lymph nodes and to distinguish normal tissue from sample where a pathological (i.e. tumor) situation is present. We find results in good agreement with literature and we present a faster analysis protocol.

Abstracts

– Live cell imaging –

P-331

Influence of 3D bleach distribution in FRAP experiments in conventional and non-linear excitation

F. Cella[1], D. Mazza[1], G. Vicidomini[1], S. Krol[2], A. Diaspro[1]
[1]LAMBS-MicroScoBio, DIFI University of Genoa, Italy, [2]CBM-Scrl Center of molecular biomedicine Trieste, Italy

The quantitative analysis of FRAP experiments usually requires the development of suitable model. When diffusion in 3D environments is considered, the description of the initial condition produced by the perturbation (i.e. the photobleaching of a selected region) represent a crucial aspect, as the approximations that are usually made can lead to deviation in the measurement of the kinetic parameters of the labeled molecules. Furthermore the experimental distribution of fluorescent molecules depend on the intensity of the light pulse that produce the perturbation as fluorescence saturation would play a role In this work we measured the experimental 3D bleaching distributions produced in conventional and two-photon excitation schemes and analyzed the deviations from the idealized cases usually adopted. The experimental measurement of these pattern for different experimental conditions in immobile samples (labeled polyelectrolyte gels) revealed that the approximation of the confocal bleaching intensity distribution as Gaussian can lead to relevant errors. On the opposite side the two-photon bleach volume seems well described by such approximation, even when fluorescence saturation effects arise.These data has been used for finite elements simulations mimicking FRAP experiments on free diffusing molecules and compared with model FRAP curves based on the idealized bleach distributions. The results show that two photon excitation provide a better fit to the idealized bleaching patterns even in fluorescence saturation regime, resulting in correct estimations of diffusion coefficients within the 20%.

P-332

Phase-sensitive Raman tweezers for biological application

A. C. de Luca, G. Rusciano, G. Pesce, A. Sasso
Università di Napoli Federico II, Dipartimento di Scienze Fisiche, Napoli, Italy

Optical tweezers (OT) allow to manipulate objects in the range between 50 nm and 100 μm. In recent years, big efforts have been made to combine OT with several spectroscopic technique such as fluorescence and Raman spectroscopy. Among this technique, Raman analysis of trapped object has revealed particularly useful, providing information concerning chemical composition and structural conformation of the investigated sample. A general problem affecting either Raman spectrometers than Raman Tweezers systems is the background contribution coming from the environment surrounding the sample. This drawback is usually overcome by subtracting the acquired spectrum from a reference spectrum. In this work we report on a novel method which allows acquiring Raman spectra of trapped particles (polystyrene microspheres)free from any background contribution and without any subtraction procedure. The method is based on the use of two collinear and co-propagating laser beams: one is devoted to trapping (trapping laser), and a second one is used to excite the Raman transitions (pump laser). The trapping laser, by means of a galvomirror, moves periodically the trapped particle back and forth along one direction perpendicular to the propagation axis at a given frequency. The scattered photons signal is sent to a lock-in amplifier for a phase-sensitive detection. The results found demonstrate that this method may find valuable applications in rapid sensing of biological samples in aqueous solutions. One of the potential applications lies in studying the diffusion of molecules into micro-sized particles.

P-333

On the correlation between tissue hydration, impedance and pain threshold in rats

A. S. Deghoyan, H. Y. Stepanyan, R. H. Simonyan, G. C. Musheghyan, S. N. Ayrapetyan
UNESCO Chair-Life Sciences International Postgraduate Educational Center, Yerevan, Armenia

The conductive characteristics of cell membrane provide the active component, acting as imperfect capacitors, contribute a frequency-dependent reactive component. Impedance measurements made over a range of low (14,5 Hz) to high (1450 Hz) frequencies, therefore it allows to predict on the extracellular fluid volume and on intracellular water volume as differences between tissues impendence measured at high and low frequencies. Previously the close correlation between cell hydration and cell membrane impedance characteristics was shown. The cell swelling led to membrane excitability and decreased the pain threshold in animals. Therefore, the aim of the present work was to find out weather the tissue impedance could serve as a marker for pain sensitivity of rat extremities to "hot plate". For this purpose the effect of distillated water drunk by rats on brain tissue hydration, pain threshold and impedance characteristics were studied. Tissue hydration was determined by wet weight/dry weight of animal tissue, pain threshold- by "hot plate" test and tissue impedance- by potentiometric method. In rats watered by distillated water during 5 days the brain cortex and skeletal muscle tissue hydration were increased by 20%, which was accompanied by the decrease of pain threshold by $\sim$20% and increase of active and reactive components of tissue impedance by 100% and 60%, correspondingly. Thus, the tissue impedance could be considered as a marker for brain and muscle tissues excitability.

P-334

Soft laser irradiation effects on human mononuclear cells mitochondrial network in stress conditions

I. O. Doaga[1], E. Radu[1], T. Seremet[1], A. Bobocea[1], E. Fertig[1], G. Katona[1], J. Horvath[2], E. Tanos[2], L. Katona[1], E. Katona[1]
[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [2]LASEUROPA, Budapest, Hungary

Searching for disclosure of sub-cellular mechanisms involved in soft laser irradiation healing effects, the present studies were undertaken to monitor short and long term changes occurring in the mitochondrial network weight and state in T leukemia lymphoblasts Jurkat, and in human peripheral blood derived lymphocytes, monocytes/dendritic cells. Near-infrared or far-red light emitted by therapeutic lasers (830nm/55mW and 680nm/25mW) and various treatment regimes (daily/every second day, single incident doses of 0.8-1.8 μJ/cell, total doses up to 15 μJ/cell) were used to irradiate the cells cultured in standard conditions, virtually alone or in co-culture, in presence/absence of cytokines/growth factors. Cultured cells were exposed to stress conditions induced by energy/nutrient restriction for various time periods (1-7 days), and the influence of presence of flavonoids (quercetin/epigallocatechin gallate) in various concentrations (1-200μM), also was tested. The confocal microscopy and flow cytometry data obtained using appropriate molecular reporters (JC1, MitoTracker Green/Red) document significant laser induced radiation dose, irradiation regime, cell type and state, and cell culture conditions dependent changes in mitochondrial membrane potential, mitochondrial network size, shape and distribution, strongly modulated by bioflavonoids' presence.
Partial financial support of the Romanian Ministry of Education and Research (grant CNCSIS 924/2006 and grant CEEX 74/2006) is gratefully acknowledged.

Abstracts

– Live cell imaging –

P-335

Tetraspanins dynamics in cell membrane using single dye tracing

C. Espenel[1], E. Margeat[1], P. Dosset[1], C. Arduise[2], E. Rubinstein[2], P.-E. Milhiet[1]
[1]INSERM, Unité 554, Montpellier, France and Université de Montpellier, CNRS, UMR 5048, Centre de Biochimie Structurale, Montpellier, France, [2]INSERM, U602, Villejuif, France and Université Paris 11, Institut André Lwoff, Villejuif, France

Tetraspanins are glycoproteins with four transmembrane domains, expressed in a wide range of tissues and cell types. These proteins have been implicated in several physiological processes (cell adhesion, migration, antigen presentation, cell activation) and pathologies. Tetraspanins have the singular particularity to organize into supramolecular assemblies at the cell surface. These assemblies, named tetraspanin web or TEMs (Tetraspanin-Enriched Microdomains), are based on the formation of primary complexes, composed of a tetraspanin and a primary partner, which could associate through tetraspanins interactions building larger complexes. Tetraspanin palmitoylation and membrane cholesterol are both involved in the formation of these microdomains.
In order to better understand the formation of TEMs and the mechanisms involved in lateral segregation of tetraspanins and their partners, we have performed single molecule analysis of the dynamics of CD9, one of the best characterized tetraspanin, using Total Internal Reflection Fluorescence (TIRF) microscopy equipped with a dual-view CCD-based detection. The membrane behaviour of wild type and non palmitoylated CD9 was investigated in the context of TEMs and raft microdomains.

P-336

Direct Vpr-Vpr interaction monitored by Förster Resonant Energy Transfer (FRET)

J. Fritz, P. Didier, E. Schaub, Y. Mély, H. Rocquigny
Photophysique des Interactions Biomoléculaires, Faculté de Pharmacie 74 route du Rhin F-67401 Illkirch

Viral Protein R (Vpr), from HIV type I virus, is a 96 amino acid protein implicated in several cellular processes during the viral cycle. Recent experiments have evidenced the role of the N-terminal part in virion incorporation, nuclear localization and Vpr oligomerization. On the other hand, the C-terminal part is involved in the G2 cell cycle arrest, apoptosis and in the interaction with the nucleocapsid protein and nucleic acids. The structure of Vpr was obtained by NMR with addition of solvent or micelles. The difficulties to obtain structural elements could be explained by the potency of Vpr to oligomerize through formation of leucine zippers. Moreover it has been shown that Vpr is able to form channels in lipid bilayers. In order to investigate the Vpr oligomerization in a cellular context a series of experiments using GFP and mCherry fusion proteins were achieved. These fluorescent proteins were used as a donor and acceptor in resonant energy transfer experiments so that by measuring the fluorescence lifetime of the donor it is possible to obtain information about interacting proteins. Time-resolved imaging was performed with a home-made two-photon laser scanning microscope. From energy transfer measurements, Vpr-Vpr interaction is shown in HeLa cells mainly at the nuclear envelop level but also in the cytoplasm and nucleus. The energy transfer depends on the position of the fluorescent protein on the N or C terminus in Vpr. Deletion or substitution of amino acid predictively involved in the Vpr tridimensional folding elicits a large decrease in energy transfer while mutation of other residues does not hamper Vpr oligomerization.

P-337

Hialuronic Acid a substitute of polymers in lowering spermatozoid motion speed for ICSI procedure

R. F. Georgescu, D. Ilie
Univ. of Medicine & Pharmacy Victor Babes, Timisoara, Romania

In the developed countries the medium age of women that became mothers has increased. It is not uncommon that women at 40 years want to have babies. That is a reason why assisted fertilisation has begun to spread. We can distinguish two principal methods: the simple in vitro fertilisation (IVF) and the intracitoplasmatic sperm injection (ICSI). In our experiment we have used an Eppendorf cell micromanipulator mounted on a reversed Nikon Microscope. The first arm of the micromanipulator is a holder or trap for ovocites and the other one is a lancet pipette for grabbing, aspirating and injecting spermatozoids. The goal of this experiment was to obtain a new medium for sperm motion lowering without the use of PVA (polyvinylalcohol). PVA is a synthetic compound, naturally not present in the human or animal body. For this reason a pair of mice was isolated in two separate cages, male and female. The female was overstimulated with hormones (hCG 2x5UI) (human Corionic Gonadotropin) for ovocite production. Ovocites were collected (metaphase II stadium) 16h after the last intraperitoneal injection with hCG. Sperm was collected from the male by castration. The sperm was washed and separated in specialised medium. Very mobile sperms have been collected. Lowering motion speed of sperms is necessary for ICSI procedure. Here is entering our new developed medium, based on Hialuronic Acid (HA) present naturally in human and animal body. The speed of sperms treated with HA is lowered about 90% compared with PVA medium. This speed is sufficient low for ICSI procedure. The concentration of Hialuronic acid in our new created medium was 0,2 %.

O-338

Tunnelling nanotubes, a novel route of cell-to-cell communication

H.-H. Gerdes
Department of Biomedicine, University of Bergen, Norway

Intercellular communication is a major requirement for the development and maintainance of multicellular organisms. Diverse mechanisms for the exchange of signals between cells during evolution have been established. These include proteinaceous channels of animal cells, called gap junctions, and intercellular membrane channels between plant cells, called plasmodesmata. Recently, we described an independent form of cell-to-cell communication between animal cells based on the formation of highly sensitive nanotubular structures mediating membrane continuity between connected cells. With respect to their peculiar structure, these membrane channels were termed tunnelling nanotubes (TNTs). TNTs could form *de novo* between cells various mammalian cell lines as well as between cells of primary cultures. TNTs are fragile, actin-rich structures, which facilitate the intercellular transfer of vesicles of endocytic origin as well as, on a limited scale, of membrane components and cytoplasmic molecules. In the presence of F-actin depolymerising drugs the intercelluar transfer of vesicles is strongly reduced. This led to the hypothesis of a novel biological principle of cell-to-cell communication based on TNT-mediated, intercellular membrane continuity and active transport of molecules. Subsequent studies showing comparable membrane channels in variegated cellular systems of animals supported this model. Moreover, these studies point to important physiological functions of these novel connections in cell-to-cell communication.
[1] Rustom, A., Saffrich, R., Markovic, I., Walther, P., Gerdes, H.-H. (2004). Science 303:1007-1010.

Abstracts

– Live cell imaging –

P-339

Cell membrane dynamics revealed by raster-scanning image correlation spectroscopy (RICS)

E. Gielen[1], M. Vandeven[2], B. de Clercq[3], N. Smisdom[2], R. Gijsbers[4], Z. Debyser[4], Y. Engelborghs[1], M. Ameloot[2]

[1]Biomolecular Dynamics, University of Leuven, Belgium, [2]Cell Physiology, Hasselt University, Belgium, [3]Eindhoven University of Technology, The Netherlands, [4]Molecular Virology and Gene Therapy, University of Leuven, Belgium

The aim of this study is to explore the membrane heterogeneity of living oligodendrocytes, i.e. the myelin-producing cells of the central nervous system. Recently we could demonstrate, using Z-scan fluorescence correlation spectroscopy (FCS), that the lipid probe DiD exhibits hindered diffusion in the plasma membrane of the OLN-93 cell line. To investigate the diffusion behavior of the myelin specific protein MOG (myelin oligodendrocyte glycoprotein), a stable OLN-93 cell line expressing MOG-eGFP was generated. The motion of MOG-eGFP, however, is too slow to be monitored by conventional FCS. Therefore, we used RICS (raster image correlation spectroscopy). In RICS, a temporal stack of images is taken with a laser-scanning confocal microscope. Spatial correlation of this series of images yields information about the molecular dynamics on different timescales and the average molecule density. Data were obtained on a Zeiss LSM 510 META one-photon confocal microscope with a 40x oil/NA 1.3 objective. Control measurements on FITC-dextrans and fluorescent beads as well as simulations were performed to validate the method and the home-made software for data analysis. RICS-analysis of the MOG-eGFP data yields diffusion coefficients of the order of 0.1 $\mu m^2/s$. The average number of MOG-eGFP molecules is a few thousand per μm^2.

O-340

Direct observation of single muscarinic acetylcholine receptors in live cells

J. A. Hern[2], G. I. Mashanov[1], A. Baig[1], J. E. Corrie[1], B. Birdsall[1], N. J. Birdsall[1], J. E. Molloy[1]

[1]MRC National Institute for Medical Research, London, UK, [2]Institute of Cell Signalling, The University of Nottingham, UK

We have used TIRF (total internal reflection fluorescence) microscopy to observe single molecules of the human M_1 muscarinic receptor expressed on living CHO cells. We labelled cells with a fluorescent antagonist, Cy3B-telenzepine which has a high affinity for the M_1 receptors (0.1nM) and slow dissociation kinetics ($t_{\frac{1}{2}}$ > 24 hours at 23°C). Our labelling protocol resulted in ca. 80% site occupancy and when the basal cell membrane was illuminated using TIRF microscopy individual fluorescent particles could be observed and their positions and intensities were recorded at a rate of 25 frames.s^{-1}. The mobility and intensities of >2,000 particles per cell could be followed for periods of up to 10s, using automated computer tracking. Plots of mean squared displacement versus dT were characteristic of unconstrained lateral diffusion (d_{lat} = 0.1 $\mu m^2 s^{-1}$ at 23°C). Nearest neighbour analysis of the objects showed no evidence of clustering or regularity in distribution. Most objects identified (>75%) had intensities consistent with a single fluorophore whilst a small proportion had intensities equivalent to 2 fluorophores, showing 2-step photobleaching, or more complex intensity level fluctuations. At the expression levels used in our live CHO cell preparations (∼1 receptor per μm^2), we found receptors did not cluster and underwent unrestricted diffusion. The majority of M_1 muscarinic receptors were labelled with a single Cy3B-Tz with occasional observations of reversible dimer formation.

P-341

Nanoscale measurement and manipulation on living cells

Y.-X. Huang

Institute of Biomedical Engineering, Ji Nan University, Guang Zhou, China

The present paper introduces our newly developed techniques for nanoscale measurements and manipulations on living cells, including the technique of establishing a micro-environment—or a micron scale bioreactor for biological cells, the 3-dimensional scanning measurement on the structure and function as well as the distribution of the molecules in a living cell.

Micro aqueous environments for biological cells with sizes of 10-20 μm in diameter were established in a drop of oil on a glass slide. Some smaller bubbles of additive solutions were also prepared and fused into the micron scale bioreactor with a cell or microbes inside, to vary its biochemical conditions. Thereafter, the distribution of the oxygenated and deoxygenated hemoglobin in an erythrocyte within the mini bioreactor was measured using a confocal laser Raman microscopy, and the rate of oxygenate of the hemoglobin was also determined by measuring the time needed for the hemoglobin transited from deoxygenated to oxygenated state, and was found to be greater in the case after the additive solution had been added. Similar measurements were also performed on the other kinds of cells. Since the micro-environment can help to keep the substances released from the cell at a measurable concentration, and keep the cell stay at a right position during the measurement of the Raman spectrum scanning, the results of the measurements can reflect more precisely the true behavior of the cell and the situation of its molecules under different conditions.

P-342

Physical description of mitotic spindle orientation during cell division

A. Jiménez-Dalmaroni[1], M. Théry[2], V. Racine[2], M. Bornens[2], F. Jülicher[1]

[1]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany., [2]Institut Curie, CNRS UMR144, Compartimentation et Dynamique Cellulaire, Paris, France.

During cell division, the duplicated chromosomes are physically separated by the action of the mitotic spindle. The mitotic spindle is a dynamic structure of the cytoskeleton, which consists of two microtubule asters. Its orientation defines the axis along which the cell divides. Recent experiments on dividing cells, which adhere to patterned substrates, show that the spindle orientation depends on the spatial distribution of cell adhesion sites. In the present work we show that the experimentally observed spindle orientation can be understood as the result of the action of cortical force generators acting on the spindle microtubules. We assume that the local activity of force generators is controlled by the spatial distribution of cell adhesion sites determined by the particular geometry of the adhesive substrate. We develop a simple physical description of the spindle mechanics, which allows us to calculate the torque acting on the spindle, as well as the energy profile and the angular distribution of spindle orientation. Our model accounts for the preferred spindle orientation, as well as the full shape of the angular distributions of spindle orientation observed in a large variety of pattern geometries. Remarkably, it also describes the transition from symmetric to asymmetric spindle orientation, observed for certain changes of the shape of the adhesive patterns. We conclude that, on the basis of a few simple assumptions, we can provide a quantitative description of the spindle orientation of adherent cells (Nature, in press).

Abstracts

– Live cell imaging –

P-343

Mobility of fluorescent Weibel-Palade body proteins in the secretory pathway of endothelial cells

N. I. Kiskin, L. Knipe, L. Hewlett, M. J. Hannah, T. Carter
MRC, National Institute for Medical Research, London, UK

Weibel Palade bodies (WPBs) are endothelial cell-specific secretory organelles containing von Willebrand factor (VWF). VWF exists in the endoplasmic reticulum (ER) as a pro-protein (Pro-VWF) that is cleaved in a late Golgi compartment producing mature VWF and its propolypeptide (Proregion). Both proteins are co-packed within WPBs forming a dense core that may include other secreted proteins, e.g. tissue plasminogen activator (tPA), Eotaxin-3. WPB membrane components include the integral membrane proteins P-selectin (P-sel) and CD63 and the membrane-associated protein Rab27a. Expression of EGFP fusion constructs of all of the above proteins fluorescently labels WPBs in cultured human endothelial cells. The mobility of WPB proteins was assessed at 22°C using FRAP methods 1) in the ER (core proteins) by following the broadening of Gaussian distribution due to diffusion; 2) in individual immature and mature WPBs (all proteins) by the analysis of mean fluorescence in bleached and unbleached WPB compartments and 3) in structures formed by WPB kiss-fusion events. In the ER Pro-VWF and a homologue lacking the propolypeptide were less mobile (0.04-0.06 $\mu m^2/s$) than Proregion (0.33$\mu m^2/s$) or tPA (0.23$\mu m^2/s$). In individual WPBs all core proteins were immobile, Rab27a was highly mobile (0.46$\mu m^2/s$), CD63 was less mobile (0.10$\mu m^2/s$), while P-sel was immobile. No differences in protein mobility were found in immature WPBs. In kiss-fusion structures VWF was immobile, other proteins showed variably increased mobilities due to secretion-related structural transformations within the WPB or interactions between WPB membrane and cytosolic components.

P-344

Optical studies of a constitutive secretory organelle of human endothelial cells

L. Knipe[1], A. Meli[1], M. J. Hannah[1], J. Dempster[2], T. Carter[1]
[1]MRC, National Institute for Medical Research, London, UK., [2]University of Strathclyde, Glasgow, UK.

Endothelial cells respond to chemical and mechanical signals in their envirnoment by releasing a wide range of biomolecules. Secreted molecules exit the cell through several pathways; for preformed peptides and proteins these include constitutivley secreted organelles (CSO) and/or secretagogue-sensitive storage organelles called Weibel Palade bodies (WPBs). Using optical techniques we have investigated the morphology, cargo, life-time and kinetics of secretogogue-evoked secretion of a CSO in HUVEC. Morphologically, CSO comprised small spherical organelles that in resting cells contained the anti-coagulant tissue plasminogen activator (tPA). Exposure of cells to IL-1 (1ng/ml, 24h) or IL-4 (20ng/ml) upregulated expression of GROα, MCP-1, IL-8 or Eotaxin-3 respectively, all of which entered CSO. MCP-1, IL-8 and Eotaxin-3 were also detected in WPB. Expression of tPA-EGFP fluorescently labeled CSO in living cells and co-localised with GROα, MCP-1, IL-8 and Eotaxin-3. In contrast to WPBs, CSO did not contain von Willebrand factor, P-selectin or CD63, and did not recruit Rab27a. Disruption of the Golgi apparatus by Brefeldin A (BFA; 5μM) lead to a loss of tPA-EGFP containing CSO in live HUVEC with a half time of 16 minutes and a complete block of constitutive release of tPA-EGFP after 1h. Under identical conditions the WPB population, and its secretability was unaffected. Histamine (100μM) evoked release of tPA-EGFP containing CSO with a delay between the histamine-evoked rise in $[Ca^{2+}]_i$ and first CSO fusion event of 4.35±3.32s, and maximum rate of fusion of 3.49± 2.27 CSO s^{-1}.

P-345

Moesin regulates cortical rigidity, cell rounding and spindle assembly during animal cell mitosis

P. Kunda[1], A. Pelling[2], T. Liu[1], B. Baum[1]
[1]Ludwig Institute for Cancer Research – UCL branch, 91 Riding House St., London, U.K., [2]London Centre for Nanotechnology, UCL, Gordon St., London, U.K.

During animal cell division, spindle assembly and chromosome segregation are accompanied by a complex series of changes in cell shape. These begin with retraction of the cell margin and cell rounding at the onset of mitosis. Cells then elongate during anaphase, before undergoing cytokinesis at the exit from mitosis. Although previous studies have implicated the actin cytoskeleton in early mitotic events, the molecular mechanisms involved remain largely unknown, and the functional significance of mitotic cell rounding has yet to be established. Here we identify a novel role for Moesin, an ERM family protein that crosslinks actin filaments to integral plasma membrane proteins, in the control of mitotic cell shape. Moesin is activated by phosphorylation at the onset of mitosis, downstream of the Slik kinase. This cortical pool of P-Moesin is sufficient to stiffen the cell cortex, causing cells to round up. As a result, mitotic cells lacking Moesin are soft and flat. Surprisingly, the loss of mitotic cortical tension also compromises the establishment of a stable metaphase spindle; a defect that can be reversed by rescuing cortical tension from the outside. These data suggest that the rigid mitotic cell cortex acts as a physical foundation upon which to establish a well-formed mitotic spindle. Then, at the onset of anaphase, P-Moesin is lost from the cell poles, enabling the spindle to elongate as the chromosomes are segregated. In conclusion, the regulation of Moesin activity and cortical tension are essential for proper animal cell mitosis, and their deregulation may contribute to an increased incidence of aneuploidy and to development of cancer.

P-346

Analysis of Actin filaments and Microtubules dynamics in neuronal growth cones

J. Laishram, D. Avossa, R. Shahapure, F. Di Fato, V. Torre
SISSA-ISAS, Trieste, Italy

Neuronal growth cones, consisting of lamellipodia and filopodia, are highly motile sensory structures at the tip of neurites, transducing guidance information into directional movements towards targets. Lamellipodia are made up of microtubules and actin filaments while the filopodia contains primarily actin filaments and only occasionally microtubules.

To follow and quantify the dynamics of these components, we performed live Confocal imaging of growth cones from two types of neurons: Dorsal Root Ganglion (DRG) neurons and PC12 cells. To determine the most efficient method for labelling microtubules and actin filaments, we explored liposome-mediated transfection, microinjection and electroporation. PC12 cells, grown in the presence of NGF to induce differentiation, were co-electroporated or co-transfected with two plasmids, one coding for the microtubule plus end tracking protein EB1-GFP and the other coding for DsRed-actin. DRG neurons were derived from 10-12 days old rats. Electroporation of cells in suspension before plating was far more efficient. Alternatively, DRG neurons grown for 48 hours in culture were microinjected with Alexa-488 Phalloidin and Rhodamine-tubulin to label actin filaments and microtubules respectively.

Time lapse recording of the growth cones was performed in xyzt at a frame rate of 200-500 msec. Offline analysis of growth cone motility provided information about the dynamics of motion of the different cytoskeletal components. 3D structure reconstruction of the growth cone was also performed to quantify actin and microtubule concentrations.

Abstracts

– Live cell imaging –

P-347

Changes in cell deformability as a novel differentiation marker

F. Lautenschlaeger[1], M. Beil[2], J. R. Guck[1]

[1]Department of Physics, University of Cambridge, Cambridge, UK, [2]Department of Int. Medicine I, University Hospital Ulm, Germany

Regenerative medicine is a promising approach to find remedies for many diseases. A prerequisite for successful strategies is the characterising and sorting of suitable stem cells (SC) from heterogeneous populations for subsequent transplantation. The common procedure consists of binding fluorescent antibodies to specific cellular surface proteins and subsequent separation of labelled cells by FACS. In contrast, we propose that the identification of stem cells through mechanical rather than molecular characteristics may offer substantial advantages in defining stem cells. During differentiation, the function of SC changes dramatically. Functional changes are reflected by changes in the cytoskeleton which is also the primary determinant of the mechanical properties of a cell. Thus, differentiation can be detected by changes in cell mechanics. We measured the mechanical properties of cells with an optical stretcher, a specific optical trap which permits the gentle, contact-free mechanical analysis of cells in suspension. As a proof of premise, we compared the deformability of a haematopoietic precursor cell line (NB-4) to ATRA differentiated NB-4 cells. Significant differences were detected. This approach allows cells to be characterized by their specific mechanical signature. We also measured the cell deformability of primary mesenchymal stem cells after consecutive population doublings in culture and found differences in the deformability in spite of constant expression of conventional stem cell markers. This suggests that mechanical deformability constitutes a more sensitive and inherent alternative to the commonly used SC surface marker.

P-348

Relevance of Fast Scanning in FRAP Experiments

D. Mazza[1], F. Cella[1], S. Krol[1], C. Usai[2], A. Diaspro[1]

[1]LAMBS – MicroScoBIO, Physics Department, University of Genova, Italy, [2]Institute of Biophysics, National Research Council, Genova, Italy

Fluorescence Recovery After Photobleaching (FRAP) is a widely known microscopy technique developed in the mid 70s for *in-vivo* and *in-vitro* quantification of diffusion coefficients of fluorescentely labelled molecules. In a typical experiment fluorescent molecules contained in a selected region are irreversibly turned off by means of photobleaching and the redistribution of the fluorescence due to the mobility of the labelled molecules is monitored. The development of suitable analytical models allows the quantification of the molecular kinetics' parameters. In this work we analyze the effects of the finite time required for bleaching with a CLSM (Confocal Laser Scanning Microscope) on the quantitative analysis of FRAP experiments regarding diffusion and binding processes. The analysis of the systematic errors that may be brought by ignoring the relevant contribution of diffusion during the bleach phase, points out the necessity for fast bleaching approaches and for models that can take into account the coupling between bleaching and diffusion. Furthermore, we illustrate how the complete quantification of fast on-binding processes via FRAP experiments requires high sampling frequency of the first part of the recovery curve and that the increase of the noise generally associated with the decrease of the scanning time does not hide the gained accuracy. Therefore these results show that the use of fast scanning or dual scanning systems could strongly extend the range and the quality of information that can be extracted from FRAP experiments.

P-349

Kinetics of histamine-evoked Weibel Palade body exocytosis in cultured human endothelial cells

A. Meli[1], M. Erent[1], V. Babich[1], L. Knipe[1], M. J. Hannah[1], J. Dempster[2], D. Ogden[3], T. Carter[1]

[1]MRC, National Institute for Medical Research, London, U.K., [2]University of Strathclyde, Glasgow, U.K., [3]Université René Descartes, Paris 5, 75006, Paris, France

The rate, concentration-dependence and extent of histamine-evoked Weibel-Palade body (WPB) exocytosis were investigated in HUVEC expressing WPB-targeted chimeras of green fluorescent protein (EGFP). WPB exocytosis was characterized by an abrupt increase in intra-WPB EGFP-fluorescence, morphological changes and release of WPB contents. The increase in intra-WPB fluorescence was due to a rise of intra-WPB pH from resting levels to pH 7.40, coincided with uptake of extracellular Alexa-647 indicating the formation of a fusion pore, and was used here to mark the time of WPB-plasma membrane fusion for kinetic studies. In Fura-2 loaded HUVEC the delays between the increase in $[Ca^{2+}]_i$ evoked by histamine and the first fusion event were 9.9±3.9s (n=6 cells) at 0.3μM histamine and 1.6±0.2s (n=15) at 100μM histamine. The maximum rates of exocytosis were 1.20±0.16 WPB s^{-1} (n=9) at 0.3μM and 3.66±0.45 WPB s^{-1} at 100μM histamine (n=15). These occurred 2-5 seconds after histamine addition and declined to lower levels over the following 20-30s. In the presence of 1.8mM external Ca^{2+} the percentage of WPBs that exocytose increased from zero at 0.1μM, to 19.63 ±13.9% (n=20) at 1.0μM and 47.8±21.7% (n=41) at 100μM histamine. The delay and maximal rate of WPB fusion were unaffected by removal of external Ca^{2+}, however late onset fusion events were abolished.

P-350

Platelets aggregation investigated by quasi-ballistic light scattering technique

S. Neamtu, C. V. L. Pop, I. Turcu

National R&D Institute for Isotopic & Molecular Technologies, Cluj-Napoca, Romania

Blood platelets are structures highly sensitive to physiological agonists as adenosine diphosphate (ADP), thrombin, platelet activating factor (PAF) etc. In recent years new techniques based on small angle light scattering and speckle size analysis were proposed to assess platelets aggregation as alternative aggregometric tools. Here we present a quasi-ballistic light scattering technique to characterize platelets aggregation induced by ADP. The method uses an effective phase function (EPF) to describe the angular spreading of light scattered by cell suspension at small angles. In order to keep the scattered process quasi-ballistic, a diluted suspension of platelets was exposed to a linearly polarized He-Ne 633 nm laser beam. The scattered light was captured off-axis in the range of 1^0-3^0 starting the ADP addition, using a monochrome CCD camera; the images were transferred in real time to a PC and converted in data matrix. The angular spreading given by the averaged light intensity profile obtained from these images was fitted with (EPF). The corresponding anisotropy parameter (which is dependent on mean radius of the aggregates) was calculated for several platelets concentration and different moments throughout the aggregation process. The effective scattering anisotropy increases during the cell aggregation. This effect can be explained by the increase of the aggregates with larger sizes contribution.

Abstracts

– Live cell imaging –

O-351

Role of neuroligin-1 oligomerization for the neuronal synapse formation probed by FLIM

S. Pautot, E. Y. Isacoff
UC Berkeley, MCB, Berkeley, California, U.S.A.

Two neuronal membrane proteins, neurexin and neuroligin, organize macromolecular protein complexes at specialized membrane domains that lead to differentiation of presynaptic transmitter release sites. It has been established that the neuronal synapse formation requires neurexin clustering. However, neuroligin is the only protein of the pair to present an oligomerization domain. Thus, it was hypothesized that neuroligin multimerization drives neurexin clustering and triggers the synapse formation. To spatially resolve the state of neuroligin oligomerization inside and outside the synaptic contact we have resorted to fluorescence lifetime imaging microscopy. We have exploited the photophysical properties of cerulean, a fluorescent protein, to assess the interneuroligin distances and decipher the molecular interactions leading to neuroligin clustering. Neurons were co-transfected with neuroligin-cerulean and post-synaptic proteins. We recorded changes in cerulean fluorescence lifetimes and correlated them with post-synaptic density as well as pre-synaptic organization. Our data show that neuroligin-1s wild type co-localize with neurexin clusters and interact strongly with each other where neurexin clusters, and post-synaptic density proteins are present. Although neuroligin-1 oligomerization mutant is enabled to trigger synapse assembly when expressed in surrogate cell, FLIM measurement show that in neurons neuroligin-1 oligomerization mutant cluster at the synapse. This clustering parallel post-synaptic density protein accumulation suggesting that the activity of neuroligin oligomerization domain can be regulated by post-synaptic density proteins.

P-352

Protein motion in asymmetric cell division characterized by two-photon scanning FCS

Z. Petrasek, P. Schwille
Biophysics group, Biotechnologisches Zentrum, Technische Universitaet Dresden, Germany

The asymmetric cell divisions in early embryonic development are preceded by the establishment of cell polarity, which is characterized by different protein composition of distinct cellular domains. The cell polarization in C. elegans embryo, a commonly studied example of asymmetric cell division, is regulated by PAR proteins. The flow of the highly dynamic actomyosin cortex, where the PAR proteins asymmetrically distribute, is also known to play an important role in the cell polarity. It is not yet fully understood how the asymmetry of the distribution of the cortex-located proteins prior to and during the first division is established and maintained, and how the proteins move and redistribute within the cortex on the molecular scale.

Here we show how the motion of labelled proteins non-uniformly distributed in the cortex of developing C. elegans embryos can be studied with two-photon scanning fluorescence correlation spectroscopy (sFCS). Circular-scanning FCS, with its single-molecule sensitivity and maximum utilization of the fluorescence signal, provides information about fast molecular dynamics and type of motion, which is too slow for standard FCS, and not resolvable with imaging. Additionally, sFCS overcomes the problems of photobleaching and low statistical accuracy commonly encountered in standard FCS with fixed measurement volume, when applied to slowly moving molecules. By using two-photon excitation one additionally benefits from the possibility of long measurement times without disturbing the embryo development.

P-353

Photobiomodulation of flavonoid effects in energy and/or nutrient restricted human T cells

M. Pislea[1], T. Seremet[1], E. Panait[1], G. Katona[1], I. O. Doaga[1], E. Radu[1], J. Horvath[2], E. Tanos[2], L. Katona[1], V. Atanasiu[1], E. Katona[1]
[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [2]LASEUROPA, Budapest, Hungary

Seeking to contribute to the understanding of molecular and cellular mechanisms involved in soft lasers and flavonoids biological effects, the present studies were undertaken to monitor short and long term changes induced in human T cells state and behavior in normal and in energy/nutrient restriction caused stress conditions. Human T cells were cultured in standard conditions, in presence/absence of various concentrations of quercetin/epigallocatechin gallate. Energy/nutrient restriction was realized by serum starvation, glucose deprivation or blockade of glycolysis/oxidative phosphorylation. We used radiations emitted by AlGaInP/GaAs lasers (680nm/25mW and 830nm/55mW), and exposed the T cell suspensions to doses and irradiation regimes of therapeutic significance (dose densities up to 60kJ/m^2). Selecting appropriate molecular probes intracellular calcium level, cell viability, proliferation rate, cell cycle progression, and percentage of apoptotic and necrotic cells were assessed by conventional, phase contrast, and fluorescence microscopy, steady-state fluorimetry and flow cytometry. The obtained data reveal significant, laser dose, cell type and cell state dependent photobiomodulation of flavonoid effects in human T cells.

Partial financial support of the Romanian Ministry of Education and Research (grant CNCSIS 924/2006 and grant CEEX 74/2006) is gratefully acknowledged.

P-354

Small angles light scattering technique applied to red blood cells aggregation process

C. V. L. Pop, S. Neamtu
National R&D Institute for Isotopic & Molecular Technologies, Cluj-Napoca, Romania

There is a great variety of experimental techniques able to capture the light scattered by cells in suspensions extending from a simple photo-detector to sophisticated devices using CCD cameras. Light-scattering techniques are often used to investigate static or dynamic properties of biologically relevant objects such as cells in suspensions or organized in tissues. Red blood cell (RBC) is the simplest biological cell used to test theoretical methods. RBCs can be easily collected, isolated and handled and have no internal macroscopic structure. The spatial pattern of light intensity scattered by RBCs in suspension is extremely complex and only partially understood. This phenomenon is related to stochastic interference of light scattered coherently by cells located at random positions, having random orientation, and moving in a complex manner in the suspension. The result is an extremely complex time-varying speckle pattern that carry encrypted a great amount of information about the RBCs structure, shape and dynamics. The main purpose of this contribution is to investigate experimentally the angular dispersion of the scattered light upon interaction with suspensions of RBCs during cell aggregation process. The angular distribution of light scattered at small angles by RBCs was also measured with high resolution. The experimental data were compared with the predictions of the theory, and the scattering effective anisotropy was quantitatively characterized. The light-scattering technique opens new possibilities for cellular aggregation researches, for example the thrombocyte aggregation process.

Abstracts

– Live cell imaging –

P-355

Structural changes of yeast cell wall cultured in different glucose concentration

M. Reiter[1], N. Hanagata[1], J. Plasek[2]
[1]Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan, [2]Charles University, Faculty of Mathematics and Physics, Ke Karlovu 5, 121 16 Praha 2, Czech Republic

Structural changes of cell wall in yeast (Saccharomyces cerevisiae) depending on glucose concentration in medium were observed using atomic force microscopy (AFM). We cultured yeasts in the media with low (0.2%) and high (2%) glucose concentration, and prepared yeast samples for AFM observation in entrapment of cells in a microporous polycarbonate filter and by air drying of cells on substrate. In the former preparation method, contact mode and lateral force measurement revealed that the cell wall surface in high glucose concentration was rougher than that of low glucose concentration. In the latter preparation method a deformation of the cell wall can be seen. Small pits (∼50 nm in diameter) of hollow-like shape have also been found along cell wall surface in some cells cultured at high glucose experiments. Although these pits may be formed by air drying process during the preparation, such structures were observed mostly on the cells from the exponential growth phase and rarely in those from the stationary growth phase.

P-356

Particle-tracking microrheology of the cell during mitosis and cortical oscillations

S. S. Rogers, T. A. Waigh, J. R. Lu
Biological Physics Group, University of Manchester, UK

We employ particle-tracking microrheology (PTM) [1] to study viscoelasticity in cultured animal cells during mechanical changes in the cell shape – here applied to mitosis and cortical oscillations. PTM offers a method of non-invasively studying the development of the cytoskeleton in different parts of the cell, by tracking the Brownian motion of endogenous lipid droplets and other organelles. Experiments are performed in an environmentally-controlled optical microscope, equipped with a Photron CCD camera capable of imaging at frame rates in excess of 100 kHz. Thermal motion dominates at timescales of ~ 0.1 s and below, while active transport and bulk flow is usually apparent at longer timescales. Displacements of organelles are measured with accuracy of ~ 1 nm in good illumination, using newly developed software [to be published]. Anisotropic local rheology is quantified with the mean-squared displacement tensor of each particle, following [2].

[1] Waigh TA. Rep. Prog. Phys. 2005; 68:685–742.
[2] Hasnain IA and Donald AM. Phys. Rev. E 2006; 73:031901–05.

P-357

Study of nuclear receptor–coregulator interactions in live cells by cross correlation spectroscopy

J. Savatier[1], S. Jalaguier[2], E. Margeat[1], V. Cavaillès[2], C. A. Royer[1]
[1]INSERM, U554, and Université Montpellier I et II, CNRS, UMR5048, Centre de Biochimie Structurale, Montpellier, France, [2]INSERM, U824, Montpellier, France

Nuclear receptors (NR) play key roles in a multiple cellular functions and are implicated in a large number of human pathologies. As such they represent major targets of drug development programs, and many of their ligands are used therapeutically. Quantitative information about their ligand-dependent interactions in live cells would greatly enhance our understanding of their function and our ability to modulate their activity.

The goal of this research is to quantitatively characterize, in live cells, the interactions between nuclear receptors (NR) and their coregulator partners with the aim of more clearly defining their functional mechanisms. We use two photon two color cross correlation spectroscopy (TPTCCCS). This approach allows for combining 2-photon 3D microscopy with correlation spectroscopy. By using interacting protein pairs expressed as fusions of two different colored fluorescent proteins, it is possible to measure quantitatively the degree of interaction between the NR and their coregulator partners in live cells. Our study focuses on the NR implicated in estrogen signalling, human estrogen receptors (ER) α and β, and their major transcriptional regulatory partners, in particular TIF2 and RIP140, in Cos-7 cells. We first constructed cerulean-ERα, cerulean-ER β, cherry-RIP140 and cherry-TIF2, tested their activity in transiently transfected cells in the presence or absence of agonist or antagonist, and analyzed their interactions by TPTCCCS.

O-358

New trends in fluorescence correlation spectroscopy

P. Schwille
BIOTECH, TU Dresden, Germany

Fluorescence Correlation Spectroscopy (FCS) has developed into a widely used technique in modern molecular and cellular biophysics. Combining single molecule sensitivity with high statistical significance, by averaging over many single molecule transits through a confocal volume, FCS is ideally suited to study protein dynamics and interactions in situ, at minimal interference with the biological system. Particularly useful is the spectral cross-correlation where more than one fluorescent species can be studied at a time, which allows to even resolve complex reaction stoichiometry in cellular processes. Major obstacles in applying FCS to intracellular phenomena are the limited lifetime of the fluorophores under the harsh excitation conditions in the focused laser beam, and the large temporal and spatial variations of processes in living systems. Here, measurement schemes employing multiple volume elements or scanning the excitation beam are often superior to the standard setup with a single fixed volume element. It can be shown that by scanning FCS, even complex dynamics in living embryos become accessible for quantitative studies on a single molecule level. Combining FCS with other single molecule techniques such as AFM, limitations regarding the spatial resolution can furthermore be overcome.

Abstracts

– Live cell imaging –

P-359

Microenvironment dependent photobiomodulation of cyanide effects in human mononuclear cells

T. Seremet[1], G. Katona[1], M. Dumitrescu[1], I. O. Doaga[1], J. Horvath[2], E. Tanos[2], L. Katona[1], E. Radu[1], E. Katona[1]
[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [2]LASEUROPA, Budapest, Hungary

Aiming to contribute to disclosure of cellular mechanisms involved in photobiomodulation, in present studies we focused on monitoring low power long wavelength laser irradiation influence on cyanide effects in human peripheral blood mononuclear cell populations and in T leukemic Jurkat cells. Therapeutic lasers were used to irradiate cells cultured in standard conditions, in presence/absence of various concentrations of sodium cyanide, with total incident doses of 2-15μJ/cell. Peripheral blood derived adherent and non-adherent mononuclear cell populations cultured in presence/absence of cytokines/growth factors, were irradiated separately or together, and further maintained in culture virtually alone or in co-culture. Selecting appropriate surface antigen markers and intracellular molecular reporters laser irradiation influence on cyanide induced changes in cell membrane properties, intracellular calcium levels, cell cycle progression, viability and cell proliferation, was assessed in various mononuclear cell populations. The data obtained by phase contrast, fluorescence and confocal microscopy, steady state fluorimetry, and flow cytometry, demonstrate significant, intoxication level, cell microenvironment reliant cellular crosstalk, laser dose and irradiation regime dependent photobiomodulation of cyanide effects on cellular signaling leading to survival/proliferation or apoptosis/necrosis of human mononuclear cells.
Partial financial support of the Romanian Ministry of Education and Research (grant CNCSIS 924/2006) is gratefully acknowledged.

P-360

Membrane nanotubes connect human T cells and allow efficient intercellular spread of HIV-1

S. Sowinski[1], C. Jolly[2], O. Berninghausen[1], B. Önfelt[3], K. Köhler[1], M. A. Purbhoo[1], C. Hopkins[1], Q. Sattentau[2], D. M. Davis[1]
[1]Division of Cell and Molecular Biology, Sir Alexander Fleming Building, Imperial College London, SW7 2AZ, UK, [2]The Sir William Dunn School of Pathology, University of Oxford, OX3 1RE, UK., [3]Microbiology and Tumor Biology Center, Karolinska Institute, Box 280, S-171 77 Stockholm, Sweden.

When human T cells make contact and subsequently part, they can remain connected by long tethers, termed membrane nanotubes, containing filamentous actin. Nanotubes up to 100μm in length were frequently observed connecting mouse lymphocytes, primary human T cells, or Jurkat T cells, their formation and lifespan dependent on the activation state and motility of connected cells. Dynamic submicrometer-scale synapses persist within T cell nanotubes or at the contacts between cell bodies and nanotubes, as observed by fluorescence and electron microscopy. Immunofluorescent staining of nanotubes between HIV-1-infected Jurkat T cells and uninfected Jurkat or primary $CD4^+$ T cells demonstrated HIV-1 Gag and Env co-localized along the nanotube and reached the previously uninfected T cells. Live cell microscopy of infectious HIV-1 expressing GFP-tagged Gag showed viral trafficking along nanotubes from infected to uninfected T cells. Thus, membrane nanotubes represent novel physical connections between $CD4^+$ T cells and can be exploited by HIV-1 for efficient cell-to-cell spread.

O-361

PA-GFP 3-D localized photo-activation and tracking in living cells

I. Testa[1], M. Faretta[2], D. Parazzoli[2], S. Barozzi[2], A. Diaspro[1]
[1]IFOM – MicroScoBIO, Physics Dpt., University of Genoa, Genoa, Italy, [2]IEO-IFOM Consortium for Oncogenomics, Milan, Italy

Photo-activatable GreenFluorescentProtein (paGFP) represents an invaluable tool for protein/cell tracking in living cells/organisms. paGFP is normally excited at near UV wavelength (405 nm), with an emission peak centered at 520 nm. Absorption cross section at 488 nm is low in the not-activated form. However, when irradiated with high-energy fluxes at 405 nm, the protein shows a dramatic change in its absorption spectra making it efficiently excitable at 488 nm. Total Internal Reflection Fluorescence Microscopy (TIRF) allows for localised 3D excitation of fluorescent molecules inside an evanescent electromagnetic field at interfaces such as cellular membranes. Optimization of the optical set up of an objective based TIRF system allowed us to demonstrate photoactivation of paGFP fused to different membrane localizing fusion proteins. Characterization of the penetration depth showed that activation is efficiently confined in the third dimension. Two-photon fluorescence microscopy removes the restriction of localization at interfaces providing optical confinement at any focal plane within the sample volume. We therefore characterized two-photon excitation and activation properties of paGFP, showing that two-photon imaging and activation is feasible in the 750-820 nm range producing a narrow confinement along the optical axis. Optically confined photoactivation can produce novel insights into the study of biophysical mechanisms such as molecular diffusion in cellular compartments.

P-362

Characterization of particles aggregation by small-angles light scattering

I. Turcu
National Institute For Research & Development of Isotopic & Molecular Technologies, Cluj-Napoca, Romania

Scattering techniques, involving neutrons, x-rays and light, are experimental procedures most broadly used to characterize particle aggregation and soft condensed matter in general. The widely accepted approach to optical aggregates sizing is based on the single-scattering approximation. However, many particulate media are optically thick, and one should account for the essential contribution of the multiple light scattering events. Recently a new theoretical model based on the effective phase function concept [1] has been reported. The theory describes in analytic terms the angular spreading of light scattered at small angles if several conditions are accomplished: i) the light scattered on a single particle is strongly peaked in the forward direction; ii) the absorption at the light wavelength is very small and iii) the optically thickness is not very high so that the quasi-ballistic character of the scattering process to be assured. The main point of the contribution is to use angular resolved data obtained from light scattering at small angles a as a diagnosis tool for cellular aggregation. If the investigated cells are well characterized from the point of view of their optical parameters the proposed procedure allows us to obtain the most relevant parameters characterizing the aggregates: the average transversal size, the average scattering cross-section and the size distribution width.
[1] I. Turcu, Effective phase function for light scattered by disperse systems – the small-angle approximation, *J. Opt.A: Pure Appl. Opt.* ***6***, 537–543 (2004). I. Turcu, Effective phase function for light scattered by blood, *Appl. Opt.* ***45****, 4*, 639-647 (2006).

Abstracts

– Live cell imaging –

P-363

Kiss-and-run is the dominant mode of exocytosis in spontaneous and stimulated peptidergic vesicles

N. Vardjan[1], J. Jorgačevski[2], M. Stenovec[1], M. Kreft[2], R. Zorec[2]

[1]Celica Biomedical Center, Ljubljana, Slovenia, [2]LN-MCP, Institute of Pathophysiology, Faculty of Medicine, Ljubljana, Slovenia

Kiss-and-run exocytosis is considered to lead to full fusion upon stimulation of vesicles containing small classical transmitters. Whether this is also the case in the fusion of peptidergic vesicles is unknown. Spontaneous peptide discharge is slower than stimulated release because of the kinetic constraints of regular fusion pore openings. To see whether slow spontaneous release also reflects a relatively narrow fusion pore, we analyzed the permeation of FM4-64 dye and HEPES molecules through spontaneously forming fusion pores in lactotroph vesicles expressing synaptopHluorin (spH), a pH-dependent fluorescent fusion marker. Confocal imaging showed that >50% of the spontaneous exocytotic events exhibited fusion pore openings associated with a change in spH fluorescence, but were impermeable to FM4-64 and HEPES. Together with membrane capacitance measurements these findings indicate an open fusion pore diameter <0.5 nm, smaller than the peptides. In stimulated cells, >70% of exocytotic events exhibited a larger, FM4-64–permeable pore (>1 nm). Capacitance measurements showed that the majority of exocytotic events in spontaneous and stimulated conditions were transient. Stimulation increased the frequency of transient events and the fusion pore dwell-time, but decreased the fraction of events with lowest measurable fusion pore. It seems that kiss-and-run is the dominant mode of exocytosis in spontaneous and in stimulated peptidergic vesicles. Stimulation prolongs the effective opening of the fusion pore and expands its subnanometer diameter to enable peptide hormone secretion without full fusion.

P-364

Diffusion-limited calcium compartmentalization of neural processes

P. Yde[1], L. B. Thomsen[2], J. Midtgaard[2], B. G. Nielsen[3]

[1]Copenhagen University, Niels Bohr Institute, Denmark, [2]Copenhagen University, Department of Medical Physiology, Denmark, [3]Technical University of Denmark, Department of Physics, Denmark

Calcium is an important intracellular messenger, connecting electrical events with biochemical processes. In the nervous system, propagated action potentials result in calcium influx in dendrites and presynaptic terminals. In this study we explore the possibility that endogenous calcium buffers restrict the intracellular diffusion range of Ca^{2+}, thus effectively creating border-free compartments within cells. From modelling studies it is estimated that the extent of these compartments is in the submicron range, which compares favourably with experiments on synaptic boutons in the cerebellar cortex. Different mechanisms for targeted activation and establishment of compartments are discussed and the overall role in connection with neuronal signalling is evaluated, especially in connection with enhancement of information processing capabilities.

Abstracts

– Ion channels: Structure and function –

P-365

Solid State NMR Studies of Bacteriorhodopsin

L. Aslimovska, K. Varga, O. Berthoumieu, A. Watts
University of Oxford, Department of Biochemistry, UK

Over the past few years, the remarkable advances in solid-state NMR (SSNMR) approaches have contributed significantly to the characterization of protein structure and function. SSNMR holds the potential for structure determination of membrane proteins which are not soluble in aqueous solution and are difficult to crystallize (Watts et al., 2005). There are only a few studies available about the optimal SSNMR sample preparation method for membrane proteins – thus various sample preparation techniques were explored to establish the optimal SSNMR preparation method for bR. Here we report initial results of structural studies of bacteriorhodopsin from H. salinarium. Sample homogeneity affects the quality of the solid state NMR spectra. Spectra of bacteriorhodopsin in purple membrane, its natural environment, and crystallised form from bicelles were compared. The effects of deuteration on uniformly-^{13}C, ^{15}N bR were investigated in the purple membranes. Optimal conditions for producing spectra suitable for spectral assignment are reported as an initial step towards structural resolution using methods which may have applicability for other membrane proteins.

Watts, A., Straus, S.K., Grage, S., Kamihira, M., Lam, Y.-H. and Xhao, Z. (2004) Membrane protein structure determination using solid state NMR. In: *Methods in Molecular Biology – Techniques in Protein NMR Vol. 278* (ed. K. Downing), Humana Press, New Jersey, pp. 403-474.

P-366

Ion channel targets in the Membrane Protein Structure Initiative

R. M. Cleverley[1], J. Kean[1], M. Imran[1], A. Mason[2], H. Bayley[2], R. C. Ford[1], J. P. Derrick[1], S. M. Prince[1]
[1]Faculty of Life Sciences, University of Manchester, U.K., [2]Department of Chemistry, University of Oxford, U.K.

As a part of the Membrane Protein Structure Initiative, a UK based consortium of researchers focussed on membrane transport proteins, we have selected prokaryotic representatives from a number of ion channel families as targets for structural study. These currently comprise glutamate gated ion channels, chloride channels, magnesium channels and mechanosensitive channels. We will present an update on our progress on these targets with a general outline of our strategy for expression, purification, functional characterisation and crystallization screening.
http://www.mpsi.ac.uk/

O-367

Structure of the fuscopeptin pore on lipid membranes

M. Coraiola[1], R. Paletti[1], A. Fiore[2], V. Fogliano[2], M. Dalla Serra[1]
[1]FBK & CNR-IBF – Unit at Trento, Povo (Trento), Italy, [2]Dip. Scienza degli Alimenti, Univ. di Napoli "Federico II", Napoli, Italy

Fuscopeptin, a lipodepsipeptide (LDP) produced by *Pseudomonas fuscovaginae*, is the causal agent of "sheath brown rot" disease of cultivated and wild *Gramineae*. This molecule is involved into the disease development. Here, we present a biophysical investigation of the interaction of fuscopeptin with natural and model membranes. Fuscopeptin was able to permeabilize erythrocytes and pure lipid membranes. The architecture of fuscopeptin pore has been further investigated by spectroscopic and electrophysiological techniques. From FTIR spectroscopy an increase in the helical content at expenses of random coil and beta-structures was detected when the peptide enters the membrane from the buffer solution. Interestingly, the presence of both alpha and 3(10) helix could be clearly distinguished in the amide I. The fuscopeptin helical fractions were inserted into the membrane with an angle of about 30 degree with respect to the perpendicular to plane of the membrane, suggesting that fuscopeptin does not lay on the membrane surface but it is inserted into the lipid core. Fuscopeptin form pores into planar lipid bilayers: channel conductance is non-linear and depends on the membrane lipids composition. Channel opening is voltage dependent and is modulated by the electrical charge of the membrane. The cationic selectivity strongly increases if negative lipids are present into the membrane. Furthermore, fuscopeptin increases the transbilayer movement (flip-flop) of a fluorescently labelled lipid in liposomes: these evidences strongly support the toroidal architecture of the pore. *Sponsored by PAT Fondo Progetti (Project SyrTox)*

P-368

Topographical analysis of TM6 in ABCB1; perturbation by site directed cross-linking

E. H. Crowley[1], J. Storm[1], S. Modok[1], I. D. Kerr[2], R. Callaghan[1]
[1]University of Oxford, Oxford, UK, [2]University of Nottingham, Nottingham, UK

Reduced intracellular drug accumulation due to the activity of the drug efflux pump ABC^{B1} is a major mechanism in resistance of cancer cells to chemotherapy. ABC^{B1} is a poly-specific transporter and the molecular mechanism of its translocation process remains to be elucidated. To understand the mechanism requires information on the regions involved in drug binding and those that couple this to nucleotide hydrolysis. The present investigation focuses on the cytosolic region of transmembrane helix 6 (TM6), which has been widely attributed with a central role in the translocation process. A series of ABC^{B1} isoforms containing a single cysteine within TM6 were constructed and the proteins purified and reconstituted. Accessibility of the cysteines to covalent modification by maleimide reagents was assessed at each stage of the catalytic cycle for each isoform. Residues at the two extremes of the TM6 region (S344-G360) were considerably more accessible than the central segment, which also failed to undergo significant conformational changes by trapping ABC^{B1} in different catalytic intermediate states. Covalent modification of this cytosolic segment of TM6 was observed to attenuate drug stimulation of ATP hydrolysis and represents an important part in coupling drug binding to ATP hydrolysis during translocation. These results support the hypothesis that TM6 emerges from the hydrophobic core of the lipid bilayer into an aqueous like environment. Furthermore local changes in the environment at the central, hinge region of TM6 are magnified at the segment extremes.

Abstracts

– Ion channels: Structure and function –

P-369

Functional characterization of a prokaryotic cyclic nucleotide-gated ion channel

A. A. Cukkemane, T. Gensch, R. Seifert, B. U. Kaupp
Forschungszentrum Jülich, Institut für Neurowissenschaften und Biophysik, INB-1, 52425 Jülich, Germany

Cyclic nucleotide-gated (CNG) channels play key roles in signal transduction of sensory neurons. Hyperpolarization-activated cyclic (HCN) channels are important transducers involving signaling pathways in cardiac and neuronal excitability. A common underlying feature of the two channel types is their ability to bind cyclic nucleotides. Both channel types harbour a C-terminal cyclic nucleotide-binding domain. Furthermore, the channels also differ with respect to their ligand selectivity and sensitivity. CNG channels select cGMP over cAMP while the HCN channels show greater sensitivity for cAMP. The molecular basis that sets apart ligand affinity and selectivity has not been directly measured but rather inferred from electrophysiological studies. Because binding and gating events are intimately coupled, it is difficult to dissect one from the other.
Here, we report ligand binding of a cyclic nucleotide-activated K^+ channel from *Mesorhizobium loti* and its isolated cyclic nucleotide-binding domain. The channel and the isolated binding domain bind cAMP with similar affinity in a non-cooperative fashion. The cAMP sensitivities of binding and activation coincide. Thus, each subunit in the tetrameric channel acts independently. The binding and gating properties of the bacterial channel are distinctively different from those of eukaryotic CNG channels.

O-370

Voltage-gated calcium channel α2δ subunits and their role as drug targets and in disease

A. C. Dolphin
Dept Pharmacology, University College London, London WC1E6BT, U.K.

Voltage-gated calcium channels consist of α1 subunits that co-assemble with auxiliary β and α2δ subunits. The α2δ subunits are mainly extracellular with a single transmembrane domain, and play an important trafficking function[1]. The mechanism of trafficking by α2δ subunits involves the Von Willebrand factor domain[1].
The drugs gabapentin and pregabalin, used in the treatment of epilepsy and neuropathic pain, bind to α2δ1 and α2δ-2 subunits. Their mechanism of action is still unclear. However we have shown that mutations in α2δ-1 and -2, that prevent gabapentin binding, reduce the ability of α2δ to enhance calcium currents[2;3].
Mutations in neuronal voltage-gated calcium channels form the basis of a number of channelopathies, both in humans and in mouse models. The spontaneously arising mouse mutant *ducky* (*du*) represents a model for cerebellar ataxia and absence epilepsy, and has a mutation in α2δ-2[4;5].
1. C. Canti et al., *Proc.Natl.Acad.Sci.USA* 102, 11230-11235 (2005).
2. A. Davies et al., *J.Neurosci.* 26, 8748-8757 (2006).
3. M. J. Field et al., *Proc.Natl.Acad.Sci.USA* 103, 17537-17542 (2006).
4. J. Barclay et al., *J.Neurosci.* 21, 6095-6104 (2001).
5. R. Donato et al., *J.Neurosci.* 26, 12576-12586 (2006).

O-371

Structural and functional characteristics of the alpha-Haemolysin channel in the hexameric form

S. Furini[1], C. Domene[2], M. Rossi[1], F. Lodesani[1], S. Cavalcanti[1]
[1]DEIS, University of Bologna, Italy, [2]Physical and Theoretical Chemistry Laboratory, University of Oxford, UK

Alpha-Haemolysin (aH) is a toxin secreted by the bacterium *Staphylococcus Aureus*, which generates nonselective pores in the membrane of susceptible cells. In recent years, aH has received great attention, mainly due to its possible usage as a sensing element for organic molecules (Li-Qun Gu et. al., Nature, 1999). X-Ray crystallography revealed a heptameric structure for aH (Song et. al. Science, 1998), while a hexameric structure was pointed out by atomic force microscopy (Czajkowshy, et. al., J. Mol. Biol., 1998). Yet, atomic detail of the hexameric structure is not available, due to the limitations of the AFM technique. The existence of these two structures is confirmed by electrophysiological data, i.e. aH in planar lipid bilayer show two distinct conductance values. The transmembrane domain of aH exhibits a beta-barrel conformation. Geometrical restraints limit the number of configurations assumed by beta-barrel channels (Murzin, et. al. J. Mol. Biol., 1994). A set of atomic models of aH in the hexameric form have been obtained by adopting these restraints. Molecular dynamics simulations have been used to validate the stability of the models. For each of the stable structures, conductance has been predicted by the Poisson-Nernst-Planck electrodiffusion theory. Finally, theoretical values have been compared to experimental data. In conclusion, according to the present results, the hexameric model with S=16 (adimensional parameter measuring the tilt of beta-sheets respect to beta-barrel axis) is the one reproducing the experimental data, not the previously proposed model with S=12 (Smart et. al., Faraday Discuss., 1998).

P-372

Myocytes are driven by constant contraction but not force upon changes in substrate elasticity.

B. Hoffmann, N. Hersch, C. M. Cesa, N. Kirchgessner, R. Merkel
Institute of Bio- and Nanosystems 4: Biomechanics, Research Center Jülich GmbH, 52425 Jülich, Germany

Muscle cells from mammalian hearts (myocytes) are embedded into a tissue changing its elasticity upon aging or in consequence of diseases e.g. a heart stroke. As a result heart muscle tissues stiffen. Interestingly, these changes in elasticity do not seem to have a major effect on the constant blood produced by the heart within a certain elasticity range. In this study we analyze if myocytes actively sense elasticity changes possibly resulting in morphological changes or if they are, in contrast to many other cell types, unaffected upon substrate stiffening. By using a two-component silicone rubber (Polydimethylsiloxane) we were able to create various substrate elasticities from 16 to 130 kPa. Such substrates were microstructured on their surface and subsequently used for myocyte morphological and cell force analysis. Our results show that heart muscle cells are morphologically unaffected by elasticity changes within the range analyzed. In addition, contraction analyses revealed almost unaffected contraction amplitudes at decreasing elasticities. At the level of forces such constant contraction amplitude resulted in increasing force formation on stiffer substrates. At substrate elastitities of around 60 kPa myocytes seem to reach their maximum cell force. This results in reduced contraction amplitudes on even stiffer substrates but further on cells applied forces at a high level. The data show that myocyte function does not depend on elasticity sensing. In fact, such elasticity independent behaviour initially enables myocytes to always contract by the same level to immediately adapt whenever changes in heart elasticity might occur.

Abstracts

– Ion channels: Structure and function –

P-373

Biochemical characterisation of the human sodium/iodide symporter (hNIS)

S. Huc, D. Marcellin, V. Navarro, T. Pourcher, E. Darrouzet, E. Quéméneur
CEA Valrhô DSV/iBEB/SBTN, 30207 Bagnols-sur-Cèze, France

Iodine, as a component of thyroid hormones, is a very important element for human physiology. Indeed, these hormones are essential for cell metabolism regulation and nervous system development. Provided in low quantities by food, iodide is concentrated at the thyroid level by the mean of hNIS. This 643 amino-acids long protein comprises 13 trans-membrane helices and its C-terminal domain, located in the cytosol, comprises the last 93 amino-acids. Its ability to accumulate iodide makes the radiotherapy of thyroid cancer possible. This property is also widely used in imaging and gene therapy could widen even more its medical application. However its biochemical characterisation is still at a very early stage.
We have established a stable cell line expressing hNIS and achieved its purification. The oligomeric state of the purified symporter has been investigated by light scattering and its functionality is studied by current recording through reconstituted planar lipid bilayer after addition of the protein. Another part of our work consists in the study of various mutants of hNIS by iodide uptake measurement of and immunofluorescence after transfection of HEK 293 cells. Besides, the C-terminal domain displays many potential phosphorylation sites and a PDZ domain recognition site. This suggests that the C-terminal part take a great place in hNIS regulation, probably through protein-protein interactions. After purification of the soluble C-terminal domain we characterised it by circular dichroïsm and NMR. These results will contribute to improve the biochemical characterisation of this essential protein.

P-374

NMR Spectroscopy of Encapsulated Membrane Proteins Dissolved in Low Viscosity Solvents

J. M. Kielec, R. W. Peterson, K. G. Valentine, A. J. Wand
Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, USA, 19104-6059

Integral membrane proteins play essential roles in a wide array of biological processes including nerve action potentials, homeostasis, cell architecture, and signal transduction. They comprise over 50% of current and potential drug targets, yet their structural characterization has lagged far behind that of soluble proteins. For solution nuclear magnetic resonance (NMR) spectroscopy, membrane proteins represent a significant challenge because their solubilization in aqueous detergent micelles results in particles of large size and attendant slow molecular reorientation. This limits the complexity of spectroscopy that can be employed. Here we will describe a novel procedure for solubilizing membrane proteins in a surfactant system suitable for dissolution in low viscosity solvents such as butane, propane, or ethane. Under these conditions, the effective tumbling of the solubilized protein is fast enough to allow for triple resonance NMR spectroscopy without the TROSY effect or the complications of extensive deuteration.. We will illustrate the approach with the homotetrameric KcsA potassium channel, which has been successfully solubilized in a hybrid reverse micelle surfactant system. Assignment quality triple resonance spectra have been obtained and progress towards the assignment of the 54 kDa KcsA potassium channel will be described.
This grant is supported by a National Science Foundation Graduate Research Fellowship to J.M.K. and The Mathers Foundation

P-375

Modulation of Mg^{2+} block of liposome reconstituted recombinant NMDA receptors by voltage and stretch

A. Kloda, D. J. Adams, B. Martinac
School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia

The NMDA receptors are ligand gated ion channels which mediate long-term potentiation, synaptic plasticity and neuro-degeneration via conditional Ca^{2+} signalling. The ionic influx through the open channel pore coincides with the presynaptic release of glutamate and postsynaptic membrane depolarization, which relieves voltage-dependent Mg^{2+} block. NMDA receptors are regulated by polymodal stimuli including fatty acids, osmotic forces, membrane phospholipids and membrane stretch. In this study we have expressed and purified NMDA receptor proteins composed of NR1a and NR2A subunits. The recombinant proteins were reconstituted into liposomes and characterised by the patch clamp technique. The protein exhibited expected electrophysiological profile of activation by glutamate and glycine and internal Mg^{2+} block. We demonstrated that the mechanical energy transmitted to membrane-bound NMDA receptor channels can be exerted directly by membrane tension developed in the lipid bilayer. Membrane stretch and application of arachidonic acid potentiated currents through NMDA receptor channels in the presence of intracellular Mg^{2+}. The correlation of membrane tension induced by either mechanical or chemical stimuli with the physiological Mg^{2+} block of the channel suggests that the synaptic transmission can be altered if NMDA receptor complexes experience local changes in bilayer thickness due to dynamic targeting to lipid microdomains, electrocompression or chemical modification of the cell membranes.

P-376

Modulation of RyRs-mediated Ca2+ signaling in submandibular acinar cells by mitochondria and ATPases

O. Kopach[1], I. Kruglikov[1], T. Pivneva[1], N. Voitenko[1], N. Fedirko[2]
[1]Bogomoletz Institute of Physiology, Kiev, Ukraine, [2]Franko National University, Lviv, Ukraine

Agonist stimulation of exocrine cells leads to the generation of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) signals driven by IP_3 receptors (IP_3Rs) that rapidly become global. In excitable cells the $[Ca^{2+}]_i$ signal triggered by IP_3Rs is propagated further by a mechanism of Ca^{2+}-induced Ca^{2+} release, mediated by ryanodine receptors (RyRs). In spite of molecular evidences showing expression of RyRs in salivary cells their role is not well known. We used microfluorimetry to measure Ca^{2+} signals in cytoplasm, endoplasmic reticulum (ER) and mitochondria. In permeabilized cells caffeine induced dose-dependent, transient decrease of $[Ca^{2+}]_{ER}$ with EC_{50}=7 mM, which was insensitive to heparin but blocked by ryanodine. However, caffeine didn't produce any rise in $[Ca^{2+}]_i$, suggesting fast local uptake of Ca^{2+} released through RyRs. Indeed, activation of RyRs produced robust mitochondrial Ca^{2+} transient. When mitochondria Ca^{2+} uptake was blocked, RyRs activation evoked non-transient increase in $[Ca^{2+}]_i$, as well as decreased in $[Ca^{2+}]_{ER}$. Upon simultaneous inhibition of mitochondrial Ca^{2+} uptake and either plasmalemma or ER Ca^{2+}-ATPase, activation of RyRs caused the $[Ca^{2+}]_i$ transient. Our data suggests that Ca^{2+} released through RyRs is mostly "tunneled" to mitochondria, while Ca^{2+}-ATPases are responsible for the initial sequestration of Ca^{2+}. Complex interplay between RyRs, mitochondria and Ca^{2+}-ATPases is supported by closely localized mitochondria with ER and plasma membranes.

Abstracts

– Ion channels: Structure and function –

P-377

Differences in the C-termini of hEag channels contribute to their differential functional expression

É. Loerinczi, C. Contreras-Jurado, W. Stuehmer, L. A. Pardo
Max-Planck-Institute of Experimental Medicine, Dept. Molecular Biology of Neuronal Signals, Goettingen, Germany

The human ether-à-go-go potassium channels hEag1 and hEag2 are highly homologous, most differences in their sequence are confined to the carboxy-termini. Importantly, only one of them, hEag1, presents oncogenic properties. In addition, their functional expression in several heterologous expression systems are observed to be different.
The present study investigates the role of the C-termini in surface expression and function of these two channels, by means of chimaeric- and split channels expressed in *Xenopus laevis* oocytes. The drawback – but in the same time also the advantage – of the latter approach is, that only the 'end product' of the whole expression-process can be assessed, i.e. when all components of the channel monomer are present.
The results show that functional chimaeric channels are formed only in the presence of the C-terminus of hEag1, but not hEag2, independent of the integrity of the entire monomer, and suggest that differences in the carboxy-termini are important determinants of various aspects of wild type channel function.

P-378

Using composite multicellular spheroids to bioprint vascular tubes

F. Marga, C. Norotte, G. Forgacs
University of Missouri - Columbia , Physics Department, U.S.A.

The Differential Adhesion Hypothesis is a morphogenetic principle that provides a framework to study tissue patterning. It states that tissues composed of adhesive and motile cells mimic liquids and possess tissue specific apparent surface tension. Our goal is to build functional living structures, such as vascular grafts, by combining tissue liquidity with the technology of bioprinting. Bio-ink particles (multicellular spheroids) were placed in bio-paper (biocompatible environment) by the use of a bio-printer (three-dimensional delivery device). Surface tension measurements of spheroids, composed of endothelial cells, smooth muscle cells and fibroblasts (the cell types encountered across the wall of a blood vessel) showed that the endothelial aggregates are the least cohesive and smooth muscle aggregates are the most cohesive. The outcome of engulfment and sorting experiments were consistent with the measured values of the tensions: (i) when two aggregates of different type were placed contiguously, fibroblasts spread over smooth muscle cells, whereas endothelial cells spread over the other two; (ii) When two types of cells were randomly intermixed into composite aggregates and allowed to sort, the more cohesive aggregate ended up always being surrounded by the less cohesive one. Both homocellular and heterocellular aggregates were printed into linear and branched tubular configurations. Postprinting self-organization resulted in the formation of solid hollow tubes. Such constructs could fulfill the crucial need for small diameter vascular grafts and provide new strategies for the vascularization of tissues for transplantation. Supported by NSF 0526854.

O-379

Mutagenesis computer experiments on ligand-gated ion channels

C. Melis[1], S. C. R. Lummis[2], C. Molteni[1]
[1]Physics Department, King´s College London (UK), [2]Biochemistry Department, University of Cambridge (UK)

Ligand-gated ion channels (LGICs) are responsible for fast synaptic transmission and are target sites for many drugs. They are activated by the binding of specific neurotransmitters, which trigger a series of conformational changes, culminating in the gating of the channels.
We have investigated, using ab initio and classical computational methods, two LGICs, 5-HT_3 and $GABA_C$ receptors, whose correspondent neurotransmitters are 5-HT and GABA respectively.
Mutagenesis experiments identified the amino acids belonging to the binding site, which are relevant for the binding of the neurotransmitter and/or for the gating of the channel. To complement and help interpret such experiments, we have performed density functional theory calculations to explore the binding of 5-HT to three tyrosines (Tyr143, Tyr153 and Tyr234) in $5HT_3R$, by evaluating the effects, purely on the binding, of a series of mutations of these tyrosines. The results show that Tyr153 performs a hydrogen bond with 5-HT, while Tyr143 does not bind to 5-HT. For Tyr234, the results suggest a novel mixed hydrogen bond/cation-π interaction with 5-HT.
We also performed classical molecular dynamics simulations of $GABA_C$ to study the effect of the mutation of Arg104 on the GABA binding. The results show that the positive charge of Arg104 is essential for the GABA binding: mutations with neutral or negatively charged amino acids like alanine and glutamic acid strongly affect the stability and the binding modes of GABA.

P-380

Magnesium-dependence of electrical and motor vesical activity

M. C. Michailov[1], U. Welscher[1], M. Wirth[1], G. V. Iyengar[2], G. Schulz[1], E. Neu[1]
[1]Inst. Umweltmedizin, Int. Council Sci. Development (ICSD) & Univ. Erl.-Nuernberg, FRG, [2]IAEA, UNO NY, Vienna, Austria

Introduction. Description of stretch dependent ionic channels in vesical detrusor myocytes and their correlation with transformation of *electrical spike* (S) into *burst-plateau* (BP) activity after stretch and their Na-, K-, Ca-dependence opened a new dimension in genito-urological physiology, pharmaco-toxicology and therapy, concerning motor activity. (Br. J. Urol. **94**: 258-9, 2004; Biophys. & Mol. Biol. **65**: 170, 1996; Eur. J. Physiol. **419**: R98, 1991 and **443**: 334, 2002; Proc. Int. Un. Physiol. Sci. **14**: 508, 1980; **17**: 529, 1989; **21**: A585, 2005/Faseb J. **18/**5). **Method** (lit. see introd.): Influence of $MgCl_2$ on electrical (intracellular; myocytes) and motor detrusor **(D)** and trigonal **(T)** activity of guinea-pig preparations (n=70, $p<0.01$). **Results.** Addition of Mg (1.2-3.6 μM) transformed BP into S, antagonized by KCl. D generates spontaneous phasic (**SPC**: 2.9±0.5/min), T - spont. tonic (**STC**: 0.3±0.1/min) contractions, increased by *stretch (3 to 50 mN).* Mg (only 12-24 μM) abolished *STC* at 3, but not at 50 mN: Contr. amplitudes were not changed upto 1.2 mM, only frequency decreased. No difference of inhibitory Mg-effect on D-ampl./frequ. of SPC (3/50 mN). $CaCl_2$ (1-4x of 2.1 mM=1x) had no effect on SPC-/STC-ampl., but potentiated over 2times the inhibitory Ca-effect on STC-frequ. (50 mN). **Conclusion.** Mg essentially participates in urinary bladder el. and motor activ., probably by Ca-/Mg-dependent oscillating enzymatic systems, leading to in-/activation of "low/high voltage and stretch channels", whereby Mg plays an important role in regulation of micturition (high trigonal in-/sensitivity, after stretch).

Abstracts

– Ion channels: Structure and function –

P-381

Influence of Ba on electrical and motor vesical activity

E. Neu[1], D. Martin[1], V. Foltin[2], E. Gornik[3], W. Seidenbusch[4], M. C. Michailov[1]

[1]Inst. Umweltmedizin c/o Int. Council Sci. Development (ICSD) e.V. & Univ. Erl.-Nuernberg, Germany, [2]Inst. Histol. & Embryol., Univ. Bratislava, Slovakia, [3]Inst. Festkörperelektronik, TU Vienna, Austria, [4]Inst. Exp.Physik, Univ. Innsbruck, Austria

Fundamental ionic and molecular mechanisms of vesical motor (patho-)physiology are till today not clear: This is a cause of low therapy efficiency of incontinence, overactive bladder, toxic/radiocystitis, etc. (Urol. **68**:78, 2006; Biophys. & Mol. Biol. **65**:170, 1986; Eur. Biophys. J. **34**:765, 2005; J. Biosci. **24**:142, 1999). Influence of Ba on electrical (intracell. recording; myocytes) and isometric motor *detrusor* **(D)** and *trigonal* **(T)** activity (guinea-pig preparations) is given. Method: lit. see introd. (n=80; p<0.01-0.001). Normal spike activity (S) of *relaxed* detrusor (**3 mN**), similar to *stretched* prep. (**50 mN**), was transformed into a burst-plateau (BP) one *after replacement* of $CaCl_2$ (2.1 mM=1x) by $BaCl_2$ (0.5-0.75x of Ca) or *addition* of Ba (but not by $SrCl_2$). Spontaneous phasic contractions of D (**SPC**: 1-5/min) were changed (Ba 2.1 mM=1x): After 0.5x Ba amplitudes/frequency increased 269/144% at 3 mN and 187/103% at 50 mN. Spont. tonic contr. of T (**STC**: 0.1-0.5/min) increased after 0.25x Ba: Ampl./frequ. 157/431% (3 mN); 172/175% (50 mN). 0.5x Ba induced strong STC in relaxed whole urinary bladder (*cystotonometry* in vitro et in toto). Ba-effect suggests a correlation of electr. BP with motor STC-activities. Also these effects support the hypothesis of existence of vesical Ca^{++}-activated K^+-stretch channels. The effects of Ba (also Li, Rb, Cs, Sr) cannot be clarified only by ionic mechanisms: Properties of atoms should play an essential role. Further investigations could open new approaches to vesical electro- and pharmacotherapy.

O-382

Mechanisms and biological roles of voltage-sensor domain proteins.

Y. Okamura[1], Y. Murata[1], Y. Okochi[2], M. Sasaki[1], T. Kurokawa[1], M. I. Hossain[1]

[1]Okazaki Institute for Integrative Bioscience, Japan, [2]National Institute for Physiological Sciences, Okazaki, Japan

Voltage-sensing phosphatase (Ci-VSP) consists of the two domains: the voltage sensor domain and the cytoplasmic phosphatase domain similar to phosphatase and tensing homolog on chromosome ten (PTEN). We have previously provided evidence that phosphatase activity of Ci-VSP is dependent on the membrane potential. In this study, we performed measurements of phosphoinositide level using GFP-based imaging by confocal microscopy and electrophysiological analysis of potassium channels. PtdIns(4,5)P_2 level, as detected by PtdIns(4,5)P2-specific PH(PLC-delta)-GFP probe, decreased upon depolarization, whereas it increased upon hyperpolarization. PtdIns(3,4,5)P_3 level as detected by PH(BtK)-GFP probe also showed similar change as PtdIns(4,5)P_2. In vitro malachite green assay showed that Ci-VSP dephosphorylates PtdIns(4,5)P2 unlike PTEN. These indicate (1) phosphatase activity of Ci-VSP turns on by depolarization and (2) PtdIns(4,5)P_2 as well as PtdIns(3,4,5)P_3 are the substrate for Ci-VSP.

VSOP/Hv1 is the voltage sensor domain protein that lacks pore domain, and exhibits activities of voltage-gated proton channels. It is unclear how this protein senses membrane potential, and where proton permeates through. We addressed roles of positively-charged residues of the fourth transmembrane segment by site-directed mutagenesis. Replacement of arginine by lysine drastically shifted conductance-voltage relationship, suggesting that geometry of arginine, rather than the charge, is important for voltage sensing.

P-383

Structural and functional features of kissper, a novel pore-forming peptide isolated from kiwi fruit

D. Picone[1], M. Tamburrini[2], S. Micelli[3], D. Meleleo[3], G. Saviano[4], T. Tancredi[2], V. Carratore[2], L. Camardella[2], E. Gallucci[3], M. A. Ciardiello[2]

[1]Dept. Chemistry, Università di Napoli Federico II, Italy, [2]IBP and ICB, C.N.R., Napoli, Italy, [3]Dip. FarmBiol, Università degli Studi di Bari, Italy, [4]Dip.STAT, Universita´ degli Studi del Molise, Italy

Kissper is a 39-residues peptide isolated from kiwi (*Actinidia deliciosa*) fruit. Its primary structureis identical to the N-terminal region of kiwellin, an allergenic protein very abundant in the edible part of the same fruit recently isolated and characterized (Tamburrini et al., 2005, Protein J. 24, 423-9). This result suggests that kissper may derive from the processing of kiwellin. Kissper has a hypothetical pI of 5.4 and does not show high sequence identity with any other polypeptide of known function. However, it displays a pattern of cysteines similar, but not identical, to those observed in some plant and animal toxins or proteins involved in processes such as defence, cellular growth regulation and differentiation. In solution, kissper showed pH-triggered conformational changes. We report here the 2D-NMR structure of the peptide in acidic environment, very close to that of the kiwi fruit. Furthermore, using electrophysiological measurements we have shown that kissper is able to interact and form channel in model planar lipid membranes made up of palmitoyl-oleoyl phosphatidylcholine. Comparative analysis of its structural and functional features suggested that kissper is a member of a new class of pore-forming peptides. The high amount of kissper found in ripe kiwi fruit and its resistance to proteolysis, together with his capacity to form channels in lipid bilayer, suggests potential effects on human health.

O-384

Structural analysis of potassium-channel-ligand complexes

O. Pongs[1], A. Lange[2], S. Hornig[1], M.-F. Eauclaire[3], S. Becker[1], M. Baldus[2]

[1]Institut für Neurale Signalverarbeitung, ZMNH, UKE Hamburg, Germany, [2]Dept. of NMR-Based Structural Biology, MPI, Göttingen, Germany, [3]Institut Jean Roche, CNRS, Marseille, France

The active site of potassium channels catalyses the transport of potassium ions across the plasma membrane, similar to the catalytic function of the active site of an enzyme. Toxins from scorpion venom inhibit potassium channel activity by binding with 1:1 stoichiometry to the outer pore region and blocking potassium conduction. The binding reaction occurs both with high affinity and high specificity. We have analysed by a combination of biochemical, electrophysiological and solid-state NMR (ssNMR) spectroscopy methods the interaction of kaliotoxin, a peptide neurotoxin from scorpion *Androctonus mauretanicus* venom, with the potassium channel KcsA-Kv1.3 in proteoliposomes. The high-resolution structures revealed that both the peptide toxin and the channel exhibit different conformations in bound versus unbound states. The conformational flexibility of the toxin may be correlated with a glycine residue in the binding site. The conformational flexibility of the potassium channel receptor site may be correlated with a decreased potassium occupancy of the selectivity filter. The data indicated that structural flexibility of peptide toxin and potassium channel constitutes an important determinant for the high specificity and high affinity of peptide toxin-potassium channel interactions. Implications of these studies for designing new therapeutic agents in potassium channel pharmacology will be discussed.

Reference: A. Lange, K. Giller, S. Hornig, M.-F. Martin-Eauclaire, O. Pongs, S. Becker and M. Baldus. (2006) *Nature* 440, 959-962.

Abstracts

– Ion channels: Structure and function –

P-385

Proteorhodopsin, a new retinal binding membrane protein: Solid State NMR and 2D Crystallography

S. Shastri[1], J. Vonck[2], W. Khuelbrandt[2], C. Glaubitz[1]
[1]J.W.Goethe University, Frankfurt am Main, Germany, [2]Max Planck Institute of Biophysics, Frankfurt am Main, Germany

Proteorhodopsin (PR) originally isolated from uncultivated γ-Proteobacterium as a result of biodiversity screens, is highly abundant ocean wide. PR belongs to the Type I retinal binding protein family. It has 7 transmembranous helices with 249 amino acid residues and a molecular weight of 27 kDa. PR is a bacterial homologue of well characterized Bacteriorhodopsin and shares 26% sequence identity. The chromophore retinal is linked to the protein via a Schiff base at position Lys 231. A pH dependent vectorality of proton transfer has been observed which raises questions about a potential role as pH -dependent regulator. However, neither its functional mechanism nor its 3D structure have been resolved so far.
To gain a deeper insight to understand structural and functional relationship of PR , we combine the potential of 2D Crystallography and Solid State NMR techniques. From over 700 2D Crystallization screens, we have obtained varying quality of crystalline patterns in more than 650 screens. We also have the first 2D crystal images and projection map at low resolution. Solid State NMR was then applied to these isotopically labeled 2D crystalline preparations using uniformly and selectively labeled labeling schemes. This approach allowed us to obtain high quality SSNMR spectra with typical ^{15}N line width in the range of 0.6-1.2 ppm.
Based on this optimal sample preparation, we shall present the first Solid State NMR spectroscopic characterization of PR in a 2D crystalline arrangement.

P-386

Free energy study of ion permeation through gramidicin

S. W. I. Siu, R. A. Böckmann
Theoretical and Computational Membrane Biology, Center for Bioinformatics, Saarland University, Germany

The pentadecapeptide gramicidin forms a cation-specific ion channel in membrane environment. Two conformations are known up-to-date: the head-to-head helical dimer (HD) and the intertwined double helical form (DH). These two conformations are favored depending on the specific conditions. The biologically active form is still a matter of debate.
In this study, the energetics of potassium ion permeation by means of the potential of mean force (PMF) of both gramicidin conformations are studied applying the GROMOS G53a6 force field. The PMF for the HD agrees well with previous computational studies [1] of this conformation applying the CHARMM force field. Comparing the energy profile of the two conformations, the DH has a significantly lower central barrier and broader binding sites at the pore entrances than the HD. The clearly identified external and internal binding sites in both conformations show the energetics and location of ion dehydration upon channel entry. Multiple ion permeation appears significantly facilitated for the DH conformation due to its opposing pore water dipole moments at the pore entrances [2].
References:
[1] T.W. Allen, O.S. Andersen and B. Roux, 2006. Ion permeation through a narrow channel: Using gramicidin to ascertain all-atom molecular dynamics potential of mean force methodology and biomolecular force fields. Biophys. J. 90: 3447-3468.
[2] S.W.I. Siu, R.A. Böckmann, 2006. Electric field effects on membranes: Gramicidin A as a test ground. J. Struct. Biol. 3: 545-546.

P-387

Giant cells obtained by electrofusion: a novel heterologeous expression system

U. Terpitz[1], D. Raimunda[2], A. Zhou[1], K. Feldbauer[1], V. Sukhorukov[3], L. Beauge[2], E. Bamberg[1], D. Zimmermann[1]
[1]Dept. of Biophysical Chemistry, MPI of Biophysics, Frankfurt, Germany, [2]Instituto M. y M. Ferreyra, Córdoba, Argentina, [3]Dept. of Biotechnology, University of Würzburg, Germany

Giant HEK293 cells (Ø 30-65 μm) and giant protoplasts of the yeasts *S. cerevisiae* (Ø 15-50 μm) and *P. pastoris* (Ø 10-35 μm) were produced by three-dimensional electrofusion in hypoosmolar sorbitol media. This large size allowed to apply both whole-cell and giant excised patch-clamp techniques. Electrophysiological measurements were made at an external osmolaritiy of 270 mOsm in HEK293 giant cells and 850 mOsm in *S. cerevisiae* electrofused protoplasts. Under these conditions the specific membrane capacitance was 1.1 ± 0.1 μF/cm^2 and 0.7 ± 0.1 μF/cm^2, respectively. When fluorescein was included in the patch-pipettes a uniform intracellular distribution of the dye was seen in the whole-cell but not in the cell-attached configurations. This excludes compartmentalisation of the giant cells and protoplasts. The homogeneity of electrofusion was additionally supported by expression of the yellow fluorescent protein YFP (as part of the fusion-protein ChR2-YFP) that showed no plasma-membrane bound fluorescence in the interior of the giant HEK293 cells. Functional expression and the electrophysiological characterisation of the light-activated cation channel Channelrhodopsin 2 (ChR2) in HEK293 cells yielded results similar to those reported in the literature. Therefore, this fusion technique can provide heterologeous expression systems suitable to perform functional studies on membrane proteins.

P-388

Presenilin 1 affects cell force generation via c-Src

D. Waschbüsch[1], N. Kirchgeßner[1], C. M. Cesa[1], R. Merkel[1], J. Walter[2], B. Hoffmann[1]
[1]Institute of Bio- and Nanosystems 4: Biomechanics, Research Center Jülich GmbH, 52425 Jülich, Germany, [2]Department of Neurology, Molecular Cell Biology, University of Bonn, 53127 Bonn

Presenilin 1 and 2 (PS) are proteases cleaving transmembrane proteins. This process frees the membrane from proteins and plays an essential role in protein turnover and signalling events. Former observations had shown an altered cell adhesion of PS1 knock out (KO) mouse embryonic fibroblasts. Therefore we analyzed the function of presenilin in cell adhesion in more detail. We found that PS1-KO were characterized by an altered morphology in focal adhesions with significantly reduced sizes compared to wildtype and PS2-KO. Since size of focal adhesions correlates with cell force application, we analyzed force formation of PS mutants by using ultrasoft micropatterned elastomer films. These experiments revealed equal force applications for wildtype and PS2-KO while PS1-KO applied forces reduced by approximately 50%. Inhibiting PS function with DAPT confirmed the PS specific effect in adhesion site and force formation. Since phosphorylation is known to be a signal for stable adhesion site formation and regulated also by activated c-Src kinase, tyrosin phosphorylation levels were analyzed. We found a decreased level of phosphorylation on focal adhesion site specific proteins in inhibitor treated wildtype cells as well as in untreated PS1-KO. Such reduced tyrosin phosphorylation is caused by diminished c-Src autophosphorylation levels as c-Src activating signal. These results align with a reduced expression level of c-Src in PS1 knock out cells. Therefore, we conclude that cell adhesion and force formation are regulated in a PS1 dependent manner by c-Src.

Abstracts

– Protein-ligand interactions –

P-389

EPR spectroscopy of vinculin tail: Comparison of structural changes induced by F-actin and lipids

C. Abé[1], U. Fock[2], F. Dietrich[4], S. Illenberger[4], K.-P. Vogel[1], H.-J. Steinhoff[1], W. Ziegler[3]

[1]University of Osnabrueck, Germany, [2]University of Bielefeld, Germany, [3]IZKF Leipzig, Germany, [4]TU Braunschweig, Germany

Vinculin is a major component of cell adhesion sites anchoring actin filaments (F-actin) to the cytoplasmic adhesion plaque. The protein consists of five alpha-helical bundle domains (Vd1-Vd5) which are involved in auto-inhibitory head (Vd1-Vd4) to tail (Vd5) interactions. At cell adhesion sites a combinatorial input of several ligands is required to release intramolecular bonds and allow vinculin activation. Furthermore, the residency time in adhesion sites of less than one minute indicates that vinculin is constantly shuttling between an inactive and active state. Acidic phospholipids and F-actin bind to Vd5 competitively and they are expected to affect significantly both vinculin activation and inactivation. The structural details for their interaction with Vd5, however, remain largely uncharacterized. We studied the Vd5 structure in presence of phosphatidylserine vesicles and F-actin by electron paramagnetic resonance spectroscopy (EPR) employing single and double cysteine-mutants which were site-specifically labelled by a commonly used paramagnetic spin label (MTSL). EPR analyses reveal distinct structural changes of the Vd5 helical bundle upon binding of F-actin and phospholipid vesicles,c respectively. These different Vd5 structures modulate head to tail interaction and will provide information on the mechanism of vinculin (in)activation.

P-390

Captosemin, a Lectin from Camptosema ellipticum Seeds: properties and unfolding/refolding studies

A. P. U. Araujo, L. S. Goto, F. H. Batista, W. Garcia, L. M. Beltramini

Instituto de Física de São Carlos, Universidade de São Paulo, São Paulo, Brazil

Lectins have been placed in a structurally diverse group of proteins that bind carbohydrates and glycoconjugates with high specificity. Found in several organisms, they are extremely useful molecules in the characterization of saccharides, as drug delivery mediators, and even as cellular surface marks. We have described and studied structural and thermodynamic properties of plants lectins. One of these studies in course is about biochemistry characterization, structural properties and unfolding/refolding studies of Camptosemin, a new lectin from *Camptosema ellipticum* seed cotyledons. Camptosemin is a tetrameric lectin, with at last two N-acetyl galactosamine-binding site, and is it able to induce MDA-MB-231 cell adhesion in vitro. Its monomer has a MW about 24kDa observed on the SDS-PAGE and mass spectroscopy. Its oligomerization state, investigated by fluorescence, circular dichroism, size exclusion chromatography (SEC) and SDS-PAGE, was temperature and pH dependent. The lectin was unfolded induced by guanidine hydrochloride and pH monitored by fluorescence emission and CD spectroscopy. This unfolding/refolding process showed a two-step process, and the refolded protein exhibited the same profile of native form by SEC and showed a positive biological activity. The results obtained confirmed the successful refolding of Camptosemin, which after adopting their native three-dimensional structures, spontaneously assembles to form tetramers. Supported by Brazilian Agencies: FAPESP, CNPq, CAPES.

P-391

Molecular dynamics of protease of different HIV subtypes complexed with commercial inhibitors

P. R. Batista, P. G. Pascutti, P. M. Bisch

Instituto de Biofísica Carlos Chagas Filho – UFRJ – Rio de Janeiro – RJ - Brasil

HIV protease (PR) is one of the major targets of anti-HIV drugs and possesses a striking tolerance to mutations with several residues associated with resistance to PR inhibitors (PI). Most studies on drug resistance have examined the B subtype, the major clade in the US and Europe, and determined several mutations conferring resistance to commercial PIs. There is little information concerning PI resistance in non-B clades, although they account to around 90% of worldwide infections. It is speculated whether those HIV variants have decreased susceptibility to PIs at baseline. We have previously published MD studies with B and non-B PRs (A, B, C and F) complexed with ritonavir, showing affinity decreases, reduction of hbonds in non-B PRs. Now, we extend these simulations more 10 ns and performed other complexed to indinavir and nelfinavir. Our results of the 10 ns MD simulations with ritonavir confirmed the previous work. However the consC seems to be more stable in the 10 ns simulation. This can be noted when we look for the h-bonds and inhibitor' RMSf results, where consC before presents lower h-bonds mean number and deviations in the flaps region. The ritonavir flexibility was greater in P1' and P1. In nelfinavir only P1' presents large deviations. Indinavir, however presents the major flexibility in P2 and P2', but not when bound to consC PR where all the groups remain very stable during MD simulation.

O-392

A new mode of recognition: an entropically favoured T-cell receptor

T. Beddoe[1], L. K. Ely[1], C. S. Clements[1], J. M. Matthews[2], A. W. Purcell[3], L. Kjer-Nielsen[4], J. McCluskey[4], J. Rossjohn[1]

[1]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia, [2]School of Molecular and Microbial Biosciences, University of Sydney, Sydney, Australia, [3]Department of Biochemistry and Molecular Biology, University of Melbourne, Melbourne, Australia, [4]Department of Microbiology and Immunology, University of Melbourne, Melbourne, Australia

The underlying basis of major histocompatibility complex (MHC) restriction is unclear. Despite the increasing number of available ternary alpha/beta T-cell receptor (TCR) – peptide-MHC (pMHC) crystal structures, there is surprisingly little evidence of a unifying theme or conserved TCR-pMHC contacts. Nevertheless, current data suggests a common thermodynamic signature dictates TCR ligation. To evalute whether this thermodynamic signature defines MHC-restriction. We have examined the thermodynamic basis of highly characterised immunodominant TCR clone LC13 interacting with its cognate pMHC ligand. The thermodynamic analysis was undertaken using both surface plasmon resonance (BIAcore) and isothermal titration calorimetry. Surprisingly, we observed this interaction to be governed by favourable enthaplic and entropic forces, which is in stark contrast to the prevailing generality. We concluded that extrinsic molecular factors, such as co-receptor ligation, conformational adjustments involved in TCR signalling or constraints dictated by higher order arrangement of ligated TCRs, might play a greater role in guiding MHC-restriction than previously appreciated.

Abstracts

– Protein-ligand interactions –

P-393

Concoord/PBSA: fast free energy prediction

A. Benedix[1], B. de Groot[2], A. Caflisch[3], R. A. Böckmann[1]
[1]Theoretical & Computational Membrane Biology, Center for Bioinformatics, Saarland University, Saarbrücken, Germany, [2]Computational Biomolecular Dynamics Group, MPI for Biophysical Chemistry, Göttingen, Germany, [3]Department of Biochemistry, University of Zürich, Switzerland

The reliable and fast computation of the free energy of proteins is crucial for structure based protein design or for protein docking algorithms. Rigorous treatments involve computationally expensive methods like free energy perturbation approaches, which are, however, incompatible with the need for fast methods. Commonly used fast methods, in turn, involve empirically derived scoring functions and usually do not include the protein flexibility. Hence, such methods are inherently limited in accuracy. We propose a physical effective energy function for a fast and quantitative estimation of the free energy of mutants - a measure for their conformational stability. The first step involves the fast generation of alternative protein conformations based on geometric considerations only (Concoord[1]), aimed at efficiently sampling the available configurational space. Subsequently, an energy function is evaluated based on physical chemistry (force field), an efficient continuum solvent approach (solution of the Poisson-Boltzmann equation, nonpolar solvation) and a quasiharmonic estimate for the conformational entropy. The correlation achieved for a test set of 582 mutants was 0.74 with a standard deviation of 1.00 kcal/mol. The main advantage of this method with respect to empirically derived scoring functions is the inclusion of full flexibility by a fast sampling of conformational space.
[1] B. L. de Groot, et.al. Proteins, 29(1997). pp. 240-251

P-394

Pathogenic bacteria, host cell invasion, instrinsically disordered proteins and tandem beta-zippers

R. Bingham[1], N. A. Meenan[1], J. R. Potts[2]
[1]Department of Biology, University of York, York, UK, [2]Depts of Biology and Chemistry, University of York, York, UK

The interaction of *Staphylococcus aureus* with the human plasma and extracellular matrix protein fibronectin triggers bacterial invasion of endothelial cells. This process has been proposed to aid haematogenous dissemination of infection and the development of infective endocarditis.
The first structures of complexes between a peptide from *S. aureus* and modules from the N-terminal domain of human fibronectin have been determined. On binding, the bacterial peptide undergoes a transition from an intrinsically disordered state, to an extended antiparallel β-strand along the triple-stranded β-sheets of the sequential modules- in a 'tandem β-zipper'. Dissection of the *S. aureus* fibronectin-binding protein, based on such structural data and using isothermal titration calorimetry, has identified six sites that bind the intact N-terminus of fibronectin with dissociation constants in the nM range. Thus the bacterial protein may be 'decorated' with fibronectin molecules, each binding through? a tandem β-zipper involving four F1 modules from fibronectin. The intrinsic disorder of the bacterial protein appears to facilitate this highly efficient formation of an extended protein-protein interface.

P-395

Insight into the mechanism of PGK catalysis by molecular modelling, SAXS and crystallography

L. Chaloin[1], C. Gondeau[1], S. T. Arold[1], J. Szabó[2], B. Flachner[2], A. Varga[2], P. Konarev[3], D. Svergun[3], D. Perahia[4], B. Roy[1], P. Lallemand[1], T. Barman[1], C. Lionne[1], M. Vas[2]
[1]UMR5236, UMR5048, UMR5625, Montpellier, France, [2]Institute of Enzymology, BRC, Budapest, Hungary, [3]EMBL Outstation, Hamburg, Germany, [4]UMR8619, Orsay, France

L-Nucleoside analogues, mirror images of the natural D-nucleosides, are a new class of antiviral and anticancer agents. In the cell, the last step of phosphorylation that leads to the pharmacologically active nucleoside triphosphate forms seems to involve the human 3-phosphoglycerate kinase (PGK). Here, we propose a structure-based explanation for the low enantioselectivity of this enzyme (poster by Gondeau et al.). The structure of human PGK, obtained by homology modelling based on the pig PGK structures, was used for docking the D- or L-forms of MgADP or MgCDP. In addition, dynamic simulations on the different ternary complexes were performed to define the large amplitude domain motions using the normal mode approach. No important changes were observed for the three main rigid body motions, but flexibility of the C-domain was affected by the nature of the bound nucleotide. In parallel, crystal structures of ternary complexes of human PGK with 3-phosphoglycerate (PG) and different nucleotides were determined and show the atomic details of differences in the binding modes of D- and L-nucleotides. The occurrence of substrate-induced hinge-bending has been detected by SAXS with both complexes of L-MgADP*PG and of L-MgADP*bPG in accordance with the catalytic competence of PGK towards the L-nucleotides.

P-396

A tethered membrane platform for protein studies

J. Chopineau[1], C. Rossi[2]
[1]Université de Nîmes, 30021 Nimes, France, [2]MPIP, 55128 Mainz, Germany

Tethered bilayer construction was developed with the help of a Doelhert experimental design[1]. EggPC/ DSPE-PEG_{3400}-NHS mixed vesicles allow the vesicle/bilayer anchoring on an amine surface via amide bonds and subsequent rupture of the vesicles lead to the formation of a tethered membrane. The presence of PEG spacer chains allows decoupling the bilayer from the surface and as a consequence the presence of an aqueous reservoir between the substrate and the bilayer. Membrane formation was developed on gold and glass surfaces, in order to demonstrate the adaptability of this concept to different substrates. The well-defined geometry of this lipid assembly is convenient to apply surface sensitive techniques such as: SPR, QCM-D, AFM and fluorescence microscopy. This membrane model proved to be powerful for investigating the calcium dependent membrane binding properties of two proteins, the Neurocalcin and the Adenylate cyclase from *Bordetella pertussis*[2]. Recently, the functional reconstitution of the Voltage Dependent Anion Channel on solid support was achieved using this biomimetic membrane platform[3].
1 Rossi C. *et al.* Surface response methodology for the study of supported membrane formation. 2007 J. Phys. Chem. B: in revision. 2 Rossi C.*et al.* 2003. Differential mechanisms for calcium-dependent protein/membrane association as evidenced from SPR-binding studies on supported biomimetic membranes. Biochemistry: 42. 15273. 3 Deniaud A.*et al*. 2007. VDAC transport calcium ions through biomimetic membranes Langmuir: 23. 3898.

Abstracts

– Protein-ligand interactions –

P-397

Effect of Methoxtrexate on DHFR fast protein dynamics: a neutron scattering study

D. Clement[2], M. Tehei[1], R. M. Daniel[2]

[1]Institut Laue-Langevin, Grenoble, France, [2]Thermophile research Unit, University of Waikato, New Zealand

Molecular motions are widely regarded as contributing factors in many aspects of enzyme function. However, if we can assign 'slow' motions occurring on the millisecond scale with specific movements involved in enzyme catalysis, the role of fast protein dynamics spanning over the pico and nanosecond timescales remain unravelled. In this respect, we have conducted a series of neutron scattering experiments where a small, specific ligand, Methothrexate (MTX), known as an effective anti-cancer drug is bound to DHFR enzyme. Since MTX has an impact on the DHFR structure, dynamics and catalysis, it represents an excellent 'informative' ligand in the linkage established between fast protein dynamics and enzyme function. Using a novel elastic neutron scattering approach, we have characterized macromolecular dynamics on a picosecond timescale. Two independent parameters, the global flexibility i.e. the mean square atomic fluctuation <u2>and the structural resilience <k>. Overall, our results demonstrate that the resilience of the system is increased and inversely the flexibility is decreased upon MTX binding. The thermodynamic signature as revealed by means of isothermal calorimetry, indicates that the binding process is largely enthalpy driven with an unfavourable entropy. We suggest that MTX can modulate actively the free energy landscape by selecting specific conformational substates involved in the kinetic pathway. Thus, the modulation of the macromolecular dynamics may reflect how specific conformations are favoured for subsequent protein function in response to the binding of specific ligand.

P-398

Coupling of folding and binding in a TPR domain

M. J. Cliff[3], M. A. Williams[4], D. Barford[2], J. E. Ladbury[1]

[1]University College London, UK, [2]Institute of Cancer Research, London, UK, [3]Present address, University of Sheffield, UK formerly UCL, [4]Present address, Birkbeck College, London UK formerly UCL

Protein phosphatase 5 (Ppp5) is one of several proteins that bind to the Hsp90 chaperone via a tetratricopeptide repeat (TPR) domain. NMR and CD spectroscopy reveal that this domain is largely unfolded at physiological temperatures, and that interaction with an MEEVD pentapeptide derived from Hsp90 stabilises a folded structure. This complex, coupled folding-binding mechanism is characterised further by its observed enthalpy change on binding (determined by isothermal titration calorimetry), which displays a markedly non-linear relationship with temperature. A nested Gibbs–Helmholtz model has been developed for the global analysis of the CD and ITC data to determine separately the thermodynamic contributions of the intrinsic folding and binding events to the overall coupled process. The analysis shows that the net effect of coupled folding on the observed affinity is small. The solution structure of a complex of the TPR domain of Ppp5 with the C-terminal pentapeptide of Hsp90 reveals that the interaction is highly dynamic, there being multiple modes of peptide binding and mobility throughout the complex. Although this interaction is of very high affinity, relatively few persistent contacts are found between the peptide and the Ppp5-TPR domain. In contrast, a consensus construct for the TPR domains is extremely stable, and comparison of the sequence of Ppp5 with this consensus reveals regions that are responsible for the dynamic nature of the structure.

P-399

Identification and characterization of new calmodulin ligands

R. Dagher, D. Bonnet, P. Villa, M. Hibert, M.-C. Kilhoffer, C. Pigault, J. Haiech

UMR 7175-LC1, Équipe Chimiogénomique et Pharmacogénomique, Strasbourg, France

Calmodulin (CaM) is a calcium binding protein that regulates a network of calcium signaling pathways. It binds to a variety of cellular proteins modulating their activities and is thus implicated in many physiological processes such as metabolism, and muscles contraction. CaM exhibits multiple conformational states for target recognition.

The aim of our work is to develop a screening assay for soluble proteins to study protein-ligand interaction using fluorescence polarization/anisotropy (FP), which is simple and direct tool to study association equilibrium when free- and bound-species implicated in the equilibrium have different rotational speeds.

Using a fluorescent chemical library (1328 lissamine-tagged compounds), we have developed a FP-high-throughput screening (FP-HTS) assay for CaM, using synthetic CaM (SYNCAM). The screening allowed us to retrieve four fluorescent probes that bind to CaM in a Ca^{2+}-dependent manner. The characterization of their binding to CaM was performed by FP-titration with specific mutants (tryptophan and electrostatic mutants). Our results show a differential binding of the probes to CaM, as a function of Ca^{2+} concentration that is due to different generated conformations.

This direct FP-HTS provides us with tool for screening the Strasbourg Academic Library (4800 compounds) by FP-competitive binding assay to isolate small specific molecules which constitute valuable research tools in deciphering Ca^{2+}/CaM signaling.

P-400

Lipid interaction networks of membrane-associated proteins

F. Dancea, M. Overduin

University of Birmingham, U.K.

A growing number of proteins are known to be recruited to biological membranes through modular domains that bind lipids and insert into membranes. Two paradigms are the FYVE domain of early endosome antigen 1 and the PX domain of the Vam7p t-SNARE, which are recruited endosomal and vacuolar membranes, respectively, by recognition of phosphatidylinositol 3-phosphate and hydrophobic insertion into the membrane bilayer. In spite of recent progress in identifying protein domains that bind biological membranes, the structural understanding of membrane docking and insertion remains limited. Different types of experiments were used to map and characterize the attachment of proteins to membrane mimicking micelles including chemical shift perturbations, intermolecular NOEs and paramagnetic relaxation enhancements induced by spin labels incorporated into micelles.

Here we present a membrane associated domain docking strategy (MADDOCK) which makes use of this data in order build 3D models of protein-micelle complexes. It utilises the experimentally derived structures of the domains and a molecular dynamics model of the micelle structure. Experimental data is incorporated as ambiguous interface restraints and/or distance restraints from the micelle centre. The docking result gives a detailed view of the binding geometry of the domains to micelles as well as of the intermolecular hydrophobic, electrostatic and hydrogen bonding interactions. The method presented here is extendable to essentially any protein-micelle complex, and can be derived using any type of experimental data that identifies membrane interacting residues.

Abstracts

– Protein-ligand interactions –

O-401

Applications and limitations of use of X-ray structures in structure-based ligand design

A. Davis
AstraZeneca R&D Charnwood, Loughborough, U.K.

Structure-based design usually focuses upon the optimisation of ligand affinity. However, successful drug design also requires the optimisation of many other properties. The primary source of protein-ligand structural information is X-ray crystallography. However there is sometimes a limited appreciation of the uncertainties introduced during the process of deriving an atomic model from the experimentally observed electron density. Uncertainties in the atomic model have significant consequences when this model is subsequently used as the basis of docking, scoring and virtual screening efforts. Docking and scoring algorithms are currently imperfect. Good correlation between observed and calculated binding affinities are usually observed only when very large ranges of affinity are considered. Errors in the correlation often exceed the range of affinities commonly encountered during lead optimisation. Validation of many academic and commercial docking and scoring packages against atomic coordinates may not be the best benchmark on which to base confidence in the algorithms used. The lecture will provide case-studies and examples from the literature and in-house experience to guide successful application of X-ray structural information to Structure-based drug design.

O-402

Evolution of quorum sensing in Gram-positive bacteria revealed by the structure of PlcR

N. Declerck[1], L. Bouillaut[2], D. Chaix[1], N. Rugani[1], L. Slamti[2], F. Hoh[1], D. Lereclus[2], S. T. Arold[1]
[1]INSERM, Unité 554, Montpellier, France and Université de Montpellier, CNRS, UMR 5048, Centre de Biochimie Structurale, Montpellier, France, [2]Unité Génétique Microbienne et Environnement (GME), INRA Domaine de la Minière, F78285 Guyancourt, France

Gram-positive bacteria use extracellular signalling peptides to coordinate essential processes such as virulence, sporulation and gene transfer through 'quorum sensing', i.e. the regulation of gene expression according to population densities. This allows coordination of the behaviour of a whole bacterial community, and might have been one of the early steps in the developement of multicellularity.
Here we show that all response regulators that function *via* direct oligopeptide recognition are evolutionarily linked. Our X-ray analysis on PlcR, the major virulence regulator of the *Bacillus cereus* group, reveals that its structure and mode of peptide recognition resemble those of the pheromone receptor PrgX from *Enterococcus faecalis*. Although PlcR and PrgX lack evident sequence similarities and regulate completely different processes in different bacterial classes, their structural homology strongly suggests their evolutionary relationship. Using structure-based phylogenetic analysis, we confirm this relationship, and extend it to all other known direct oligopeptide quorum sensors. Our results elucidate the origin and evolution of multicellular behaviour in bacteria, and reveal that fundamentally different processes in different bacterial classes are regulated by essentially the same mechanism.

P-403

Nucleotide and cation binding to the sodium pump studied with biophysical techniques

M. Esmann[1], N. U. Fedosova[1], D. A. Middleton[2], D. Marsh[3]
[1]University of Aarhus, Aarhus, Denmark, [2]University of Liverpool, Liverpool, United Kingdom, [3]Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany

Active transport of Na^+ and K^+ across the plasma membrane is performed by the Na, K-ATPase through a number of structurally and kinetically distinguishable protein conformations. E_1 has a high affinity for ATP and ADP, and E_2 has K^+ firmly bound and a low affinity for nucleotides. Time-resolved and equilibrium nucleotide binding experiments reveal a large electrostatic component. At pH > 7, the affinity is lowered compared to pH 7, for both ATP and ADP. At acidic pH, ATP binds markedly better than ADP. Alkalinization leads to an increased dissociation rate constant for ATP and a decreased dissociation rate constant for ADP, suggesting that bound ATP and ADP have very different chemical surroundings. For both nucleotides, the association rate constant has its maximum at neutral pH. Na^+ and K^+ are strongly competitive, probably through occupation of the specific ion binding sites. Solid-state NMR analysis of ^{13}C-labelled ATP bound at +4 ^{o}C suggests that C8 of the adenine ring and C2, C4 and C5 of the ribose are interacting strongly with the binding site, under dynamic equilibrium conditions of binding. In the absence of molecular motion (at -25 ^{o}C), perturbation of carbon atoms of the bound nucleotide by the Na,K-ATPase is also observed. ESR spectroscopy of Mn^{2+} bound to a putative Mg^{2+} site is done at -196 ^{o}C. The fine structure in the ESR spectra suggests that bound Mn^{2+} coordinates octahedrally with predominantly oxygen ligands.

P-404

What effects do small cosolutes have on biopolymers?

M. V. Fedorov[1], I. M. Socorro[1], J. M. Goodman[1], S. Schumm[2]
[1]Unilever Cambridge Centre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, CB2 1EW, Cambridge, UK, [2]Unilever Food and Health Research Institute, Unilever R&D Vlaardingen, Olivier van Noortlaan 120 3133 AT Vlaardingen, The Netherlands

The dehydration and re-hydration of biomaterials is a central aspect of food science. A better understanding of these phenomena should lead to better processes for creating Unilever products. Cosolvents and cosolutes strongly influence the structural and thermodynamic properties of biopolymers, including effects on biopolymer solubility, biopolymer denaturation, enzyme activity, biopolymer swelling, growth rates of bacteria, and the stability of protein macroaggregates.
In this work we aim to gain insight on the underlying molecular mechanisms of salt and sugar effects on the conformational stability of biopolymers with the use of modern fully atomistic molecular dynamics simulations techniques. Such *in silico* approach allows one to obtain very realistic data on the time and scale resolutions that are unavailable for both *in vitro* and *in vivo* experimental techniques.
Our results show that for any given secondary structure of a macromolecule one can find its own 'breakers' and 'stabilizers', knowing basic principles of ligand – water, ligand – ligand and ligand – molecule interactions.

Abstracts

– Protein-ligand interactions –

P-405

Mean lifetime and first-passage time of the enzyme species involved in an enzyme reaction

M. E. Fuentes, E. Arribas, E. Valero, M. I. González, M. García-Moreno, R. Varón
Escuela Politécnica Superior, Albacete, Spain.

Taking as starting point the complete analysis of mean residence times in linear compartmental systems performed by Garcia-Meseguer et al. [1] as well as the fact that enzyme systems, in which the interconversions between the different enzyme species involved are of first or pseudofirst order, act as linear compartmental systems, we hereby carry out a complete analysis of the mean time that the enzyme molecules spend as part of the enzymatic species, forms or groups involved in an enzyme reaction mechanism. The formulas to evaluate these times are given as a function of the individual rate constants and the initial concentrations of the involved species at the onset of the reaction. We apply the results to unstable enzyme systems and support the results by using a concrete example of such systems. The practicality of obtaining the mean times and their possible application in a kinetic data analysis is discussed.

Reference
1. Garcia-Meseguer M.J., Vidal de Labra J.A., Garcia-Moreno M., Garcia-Canovas F., Havsteen B.H., Varon R., Mean residence times in linear compartmental systems. Symbolic formulae for their direct evaluation, Bull.Math.Biol. 65 (2003) 279-308.

Acknowledgements: Projects PAI-05-039 (JCCM, Spain) and BQU2002-01960 (MEC) supported this work.

P-406

The structural basis of TtgR mediated efflux pump regulation

M.-T. Gallegos[1], Y. Alguel[2], C. Daniels[1], C. Meng[2], W. Terán[1], T. Krell[1], J. L. Ramos[1], X. Zhang[2]
[1]Departamento de Protección Ambiental, EEZ (CSIC), Granada, Spain, [2]Centre for Structural Biology, Imperial College London, UK

A major mechanism of antibiotic resistance in bacteria is the active extrusion of toxic compounds through membrane bound efflux pumps that are often transcriptionally regulated. TtgR represses the transcription of TtgABC, a key efflux pump in *Pseudomonas putida* DOT-T1E, which is highly resistant to antibiotics, solvents and toxic plant metabolites. Previously we showed that TtgR binds to different classes of natural antimicrobial compounds, which are also extruded by the efflux pump. We have obtained the structures of TtgR in complex with common antibiotics and plant secondary metabolites showing that TtgR contains two distinct and overlapping ligand binding sites. Phloretin, a plant antimicrobial, can bind to both binding sites with distinct binding affinities and stoichiometries. Results on ligand binding properties of native and mutant TtgR proteins using isothermal titration calorimetry (ITC) confirm the binding affinities and stoichiometries, and suggest a potential positive cooperativity between the two binding sites. The importance of residues in both the DNA and ligand binding pockets of TtgR was also investigated using ITC and electrophoretic mobility shift assays; the results indicate the involvement of residues from both domains in the regulation of efflux pump expression. These data highlight the importance and versatility of regulatory systems in bacterial antibiotic resistance and open up new avenues for novel antimicrobial development.

P-407

Biosensing via structured interfaces at nanoscale

M. Gheorghiu, S. David, C. Polonschii, E. Gheorghiu
International Centre of Biodynamics, www.biodyn.ro, Bucharest, Romania

Surface functionalization and nanopatterning ad the required selectivity and specificity to the otherwise nonspecific, yet powerful methods like electrochemical and surface plasmon resonance (SPR) assays for biosensing. In the same time, it can provide signal enhancement, background reduction and controlled interaction with biological systems.

We will present some of our latest developments on harvesting the enhancing capabilities of nanogold (thin coating and nanobeads) plasmonics in conjunction with affinity compounds for biosensing: biosensing platforms development, modeling, data analysis and combined (electrochemical and optical) analysis set-ups, evolving to open new combinations of methods with improved technical performance, helping to resolve challenging bioanalytical problems including sensitivity, signal resolution and specificity by interfacing these technologies in small volumes. Methods Multichannel, differential impedance spectroscopy either alone or in combination with complementary methods: surface plasmon resonance (SPR), total internal reflection fluorescence microscopy, electrochemistry to develop, optimize and analyze specific nanostructured platforms (affine and cell based biosensors).

Selected results dealing with detection of target compounds and pathogens, surface interaction / and cell adhesion control through nanopatterning will be presented.

The capabilities of combined electro-optical methods to study nanostructured biointerfaces and possible applications in life sciences will be outlined.

P-408

Phosphatidylinositol transfer proteins: Membrane interaction regarding lipid composition

N. Goehring
Imperial College, London, United Kingdom

Phosphatidylinositol transfer protein α and β (PITPα/β) are soluble proteins that have the property of associating with membranes transiently to selectively extract and thus promote the exchange of phosphatidylinositol and phosphatidylcholine between lipid bilayers. PITPs are required for several cellular functions such as phospholipase C mediated signalling, regulated exocytosis and secretory vesicle formation and play a central role in lipid metabolism. However, their molecular mechanisms that regulate the rate and specific targeting of each PITP to a specific location within the cell remain unexplored. It is therefore investigated whether the curvature and the membrane composition-dependent local micro-mechanical stresses stored therein may be responsible for regulating PITP membrane association. Recombinant protein and some mutations that increase or decrease membrane association are analysed for lipid transfer and membrane binding using fluorescence microscopy and supported bilayer composition arrays within microfluidic devices.

Abstracts

– Protein-ligand interactions –

P-409

Gene expression noise in a cell is related to fluctuations in the occupancy of operator by repressor

M. V. Golovkin, Y. D. Nechipurenko, G. V. Gursky
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.

Recently new methods have been developed which enable one to measure the rate of a reporter gene expression in a single bacterial cell as a function of concentration of a gene regulatory protein, such as lambda phage CI repressor. The function is coined as the gene regulation function (GRF) by Rosenfeld et al. Intracellular concentrations of the reporter protein and modified fluorescent CI repressor can be measured simultaneously. Interaction of lambda phage CI repressor with its target operator sites on DNA is well studied *in vitro*. The affinity constants of the repressor to the operators sites O_R1, O_R2 and to their mutant counterparts are determined. The binding of the repressor to the operator sites O_R1 and O_R2 is known to be a cooperative process for which interaction between bound repressor molecules is responsible. In the present communication, a statistical mechanical approach is developed for description of gene activity regulation by lambda CI repressor. Equations are derived for evaluation of GRF for this system. The stochastic nature of the repressor–DNA binding process can be revealed when both reactants are present in a cell in small number of copies. Under these conditions, the number of the repressor molecules bound to the operator sites O_R1 and O_R2 fluctuates. Using the affinity constants and cooperativity parameter for binding of the repressor to the operator sites from experiments in vitro, we found that calculated GRF fits well with the experimental data of Rosenfeld et al. In addition, the most part of the gene expression noise observed by Rosenfeld et al is related to a stochastic nature of the binding interaction between the repressor and DNA.

P-410

Learning from peptides: Molecular Dynamics of Falcipain-2 complexed with natural peptide substrates.

D. E. B. Gomes[1], P. D. S. F. Celestino[2], P. G. Pascutti[1]
[1]Universidade Federal do Rio de Janeiro, Brazil, [2]Universidade do Rio de Janeiro, Brazil

Malaria remains one of the most important infectious diseases in the world. A potential target to new treatment is the cysteine protease Falcipain-2 (FP2), one of the major enzymes of P.falciparum. The nonspecific inhibition of FP2 lead to parasite inviability. Although the structural basis of FP2 substrate binding has already been determined, the source of the experimentally observed substrate selectivity cannot be completely understood on the basis of the static picture of crystal structures. It is proposed that the dynamic nature of proteins have an essential function in catalysis. There are two main challenges in this work: one is to define the binding mode of many peptide substrates to FP2, the other is to unravel the intimate linkage between protein flexibility and enzymatic function, investigating how the intrinsic plasticity of FP2 influences substrate recognition and catalysis, provinding insights to lead into rational drug design. To investigate this we docked ten natural small-peptide substrates with previously known binding constants to the crystal structure of FP2 (1YVB) and performed Molecular Dynamics Simulations (MD) (10ns/310K/1atm/NPT). We measured residue-specific protein essential dynamics and monitored the protein-ligand interactions elucidating the binding mechanism. Our comparative study of theoretical activity, structure and dynamics indicates not only the factors that determine the FP2 binding to such different substrates but also revealed there may be a close link between protein dynamics and catalytic turnover.

P-411

The role of calcium during fibrinogen-erythrocyte interaction

S. Gonçalves, N. C. Santos, C. Saldanha
Instituto de Biopatologia Química, IMM, faculdade de medicina de Lisboa, Lisboa, Portugal

A number of events such as those involving cellular recognition, metabolism, signaling and pathologic conditions occur in the surface of the erythrocyte membrane. Many of these processes are highly specific and can be translated as ligand receptor interactions. The spatial disposition of the electrostatic potential of the cell surface in human cells can be measured using the fluorescence probe FPE. Changes of the electrostatic surface potential will affect the protonation state of the FPE leading to changes on fluorescence intensity, as a result of the ligand interaction with the receptor. Fibrinogen is a blood plasma protein that plays a crucial role in hemostasis. Several vascular pathologies are associated with increased fibrinogen concentration, leading to alterations on erythrocyte aggregation. In the present work we used FPE as membrane label to follow the electrostatic potential changes during fibrinogen-human erythrocyte membrane interaction by using fluorescence spectroscopy. The interaction was studied by increasing the fibrinogen concentration (0.033-1.20 g/L). The results obtained clearly show that an interaction occurs between fibrinogen and the erythrocyte membrane. Previous studies suggest that the N terminal region of fibrinogen contain two crucial Ca2+-binding sites, which possibly govern the conformational changes of the molecule. In this way, interaction in the absence and presence of small concentrations of Ca2+ were performed. The results showed that fibrinogen erythrocyte interaction is enhanced by the presence of calcium.

P-412

Metabolism of antiviral drugs: Phosphorylation of D and L-nucleotides by 3-phosphoglycerate kinase

C. Gondeau[1], A. Varga[3], J. Szabó[3], B. Flachner[3], L. Chaloin[1], B. Roy[2], P. Lallemand[1], T. Barman[1], M. Vas[3], C. Lionne[1]
[1]UMR5236 CPBS, [2]UMR5625, Montpellier, France, [3]Institute of Enzymology, BRC, Budapest, Hungary

L-Nucleoside analogues, mirror images of the natural D-nucleosides, are a new class of antiviral and anticancer agents. Upon entry into the cell, nucleosides are phosphorylated by a cascade of kinases to the corresponding mono-, di- and finally, the pharmacologically active triphosphates. The last step of the cascade remains largely unexplored. This may involve kinases of low specificity for the substrate as nucleoside diphosphate kinase, creatine kinase and in particular, 3-phosphoglycerate kinase (PGK). PGK is a glycolytic enzyme that catalyses the reversible phosphotransfer: 1,3-biphosphoglycerate + MgADP ↔ 3-phosphoglycerate (PG) + MgATP. It is a typical two-domain hinge-bending enzyme which exhibits low enantioselectivity towards its nucleotide substrates. The aim of the present work is to characterize the molecular mechanisms involved in the phosphorylation of D- and L- nucleotides by human PGK. MgADP and MgCDP in their D or L conformations were chosen as purine and pyrimidine models, respectively, before extending the analysis to therapeutic analogues. We compared the formation of PGK binary complexes with the 4 different nucleotides and the effect of PG. We characterized the ligand binding properties in equilibrium and transient kinetic studies (stopped-flow) by monitoring the variation of intrinsic fluorescence of PGK or that of extrinsic probes. The phosphorylation reaction was analysed by steady state and transient kinetic experiments. The results are explained on structural basis (poster by Chaloin et al.).

Abstracts

– Protein-ligand interactions –

P-413

Correlation between thermostabilities of IPMDHs and their unfolding rates

É. Gráczer[1], A. Varga[1], I. Hajdú[1], B. Melnik[2], G. Semisotnov[2], P. Závodszky[1], M. Vas[1]

[1]Institute of Enzymology, BRC, HAS, Budapest, Hungary, [2]Institute of Protein Research, RAS, Pushchino, Russia

The relationship between conformational stability and the rates of unfolding and refolding is still an open issue. The time courses of unfolding and refolding of thermophilic *Th. thermophilus*, mesophilic *E. coli* and psychrotrophic *Vibrio* sp. I5 isopropylmalate dehydrogenases (IPMDHs) were followed by using far UV CD and fluorescence spectroscopy as well as by testing enzyme activity and the accessibility of buried thiol-groups. Unfolding in 8.5 M urea uniformly occurs as a single first order step, but its rate largely increases in the order of thermophilic, mesophilic and psychrotrophic variants: the half-times are about one hour, several minutes and few seconds, respectively. Among the substrates only the manganase complex of 3-isopropylmalate slows down appreciably the rates of unfolding: the smaller is the protein stability, the larger is the protection. Thus, denaturation of the enzyme-substrate complexes occurs in comparable time scales for the three enzymes. Unlike unfolding, refolding is a kinetically complex first order process indicating formation of folding intermediate(s). Rates of refolding are similar for all three IPMDHs, which can be attributed to formation of the specific interactions among the conserved residues, characteristic of the native IPMDH tertiary structure. Restoration of the compact native structure and of enzyme activity takes place uniformly with half time of few minutes. Taken together, different thermal stabilities of IPMDHs are not related to their similar refolding rates, but are rather due to their different unfolding rates.

P-414

Thermal adaptation of enzymatic reactions is driven by conformational fluctuations

I. Hajdú, A. Szilágyi, J. Kardos, P. Závodszky

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary

Life is adapted to a wide range of temperatures from the cold of polar regions to the heat of thermal springs. Enzymes exhibit a relatively narrow optimal working temperature range adjusted to the growth temperature of the organism. Three variants of 3-isopropylmalate dehydrogenase (IPMDH) were used to investigate the effect of temperature on the catalytic mechanisms. Nonlinear Arrhenius plots indicate the kinetic complexity of the enzyme-catalyzed reaction. The van't Hoff plots for the enzyme-substrate Michaelis constants are highly nonlinear, revealing a temperature dependent change in the mechanism of substrate binding. We hypothesized that these observations could be related to temperature dependent changes in the conformational flexibility of the protein. Backbone flexibilities were measured by amide H/D exchange experiments while relative domain-domain and subunit-subunit motions were characterized by fluorescence resonance energy transfer measurements. Both measurements indicated alterations in the nature and range of the fluctuations with increasing temperature. The stability and conformational flexibility of binary and nonfunctioning ternary complexes demonstrate the different modes of substrate and coenzyme binding. The changes in substrate binding, which require the proximity of both subunits of the enzyme, are correlated with the changes in the conformational fluctuations. This observation supports the idea that conformational fluctuations have a significant role in the catalytic function by regulating enzyme-ligand dynamic interactions.

P-415

Neurotensin Receptor 1: E.coli expression, purification, characterisation and biophysical studies.

P. J. Harding[1], J. Koeppe[1], H. Attrill[1], S. Ross[1], A. N. Kapanidis[2], A. Watts[1]

[1]Dept. of Biochemistry, University of Oxford, OX1 3QU, U.K., [2]Dept. of Physics, University of Oxford, OX1 3PU, U.K.

Neurotensin (NT) is an endogenous tridecapeptide neurotransmitter found in the central nervous system and gastrointestinal tract. One receptor for NT, NTS-1, belongs to the G-protein coupled receptor (GPCR) superfamily, has seven putative transmembrane (TM) domains, and is being studied by a range of single molecule, functional and structural approaches.

To enable biophysical characterisation, sufficient quantities of the receptor must be expressed and purified in active form. To this end, rat NTS1 has been expressed in *E.coli*, in an active, ligand-binding form at the cell membrane. Active receptor has then been produced in sufficient amounts for structural biology studies either with or without fluorescent protein (CFP and YFP) fusions. Ligand-binding was demonstrated by conventional radioligand (^{3}H-NT) binding and by a novel SPR approach using tagged ligand immobilised at the sensor surface with the detergent-solubilised receptor as the analyte. Competition binding assays using this SPR approach are now under investigation. NTS1 tagged with the fluorescent proteins can be used to optimise expression and purification through monitoring by in-gel fluorescence. Fluorescence measurements were also used to determine the oligomerisation state of the receptor which was reconstituted into *in vitro* lipid systems. Electron microscopy shows that the detergent cocktail devised produces a monodispersed receptor population suitable for further studies.

P-416

Interaction of cholate with immobilized human serum albumin investigated by FTIR ATR spectroscopy

N. Hassler, G. Reiter, D. Baurecht, U. P. Fringeli

Institute of Biophysical Chemistry, University of Vienna, Austria

FTIR attenuated total reflection (ATR) spectroscopy is increasingly used for investigations of processes at or near a surface. Very sensitive techniques are needed, particularly when biomembranes, monolayers, and thin films are studied with respect to surface concentration and molecular structure. Cholate belongs to the bile acids and is a ligand of human serum albumin (HSA). HSA plays an important role in the human organism and is, e.g. responsible for the maintenance of blood pH. In this application the interaction of cholate with adsorbed HSA was investigated. For this purpose two techniques were used; single beam sample reference (SBSR) spectroscopy and concentration modulated excitation (c-ME) spectroscopy, resulting in an optimum background compensation and signal to noise ratio. C-ME spectroscopy was applied to immobilized HSA which reacted reversibly to periodic changes of the concentration of cholate. The response of the system was measured with time-resolved FTIR spectroscopy and then processed by a phase-sensitive detection (PSD) thus eliminating disturbing signal components which did not have the same frequency like the excitation itself. The achieved sensitivity was in the micro-absorbance range, a prerequisite for studying specific interactions on a molecular level. At 5.0mM bulk concentration of cholate, one immobilized HSA turned out to bind (6.0 ± 0.2) cholate molecules. Furthermore an increase of alpha-helical structures of HSA was observed upon cholate binding. Special attention was paid to background compensation in order to separate overlapping spectral contributions of dissolved and HSA-bound cholate.

Abstracts

– Protein-ligand interactions –

O-417

Dynamics and thermodynamics of ligand-protein interactions

S. W. Homans
Astbury Centre for Structural Molecular Biology, University of Leeds, LS2 9JT

All biological processes depend on specific recognition between molecules with carefully tuned affinities. Despite the universal nature of these interactions, it is still very difficult to exploit routinely high-resolution structural data for a given complex in order to design molecules that inhibit binding. In other words, it is not trivial to predict binding affinity from structure. In the face of the emergence or re-emergence of diseases such as the relentless progress of antibiotic resistant bacterial strains, the ability to design novel ligands at will that inhibit biomolecular interactions remains one of the major challenges in contemporary science. While the crystal or NMR structure of a protein is unquestionably thought provoking in the process of ligand design, the key to understanding the affinity of a ligand for its receptor lies in the dynamics and thermodynamics of the association rather than a simple static picture. With technologies such as isothermal titration calorimetry (ITC), it is possible to obtain reliable experimental data on the global thermodynamic binding parameters. However, from the point of view of ligand optimisation, it would be of immeasurable benefit to obtain these thermodynamic parameters experimentally on a per-residue, rather than global basis. This presentation will describe recent results using NMR , ITC, protein crystallography, computational chemistry and site-directed mutagenesis in order to delineate the thermodynamics of ligand-protein associations in model systems. Particular emphasis will be placed on the decomposition of binding thermodynamics into contributions from the protein, ligand and solvent.

O-419

Protein folding coupled to binding or autoproteolysis

T. Härd
Dept. of Medical Biochemistry, Göteborg University, Göteborg, Sweden

This lecture will give examples of how protein folding can be energetically coupled to protein function. The first example involves engineered Affibody binding proteins, which are selected by phage display to be used as alternatives to monoclonal antibodies. NMR and calorimetric studies illustrate how the seemingly simple structural topologies of Affibody binders may conceal a bewildering complexity that arises when coupled folding/binding and induced fit operate simultaneously in molecular recognition. On-going studies of a complex beween an Affibody and the Alzheimer beta peptide, which involves some surprising structural transformations, will also be described. The second example of coupled protein folding and function concerns the SEA domain of the human MUC1 transmembrane mucin. NMR and biochemical studies show that this domain undergoes a novel type of auto-proteolysis, which is catalyzed by conformational strain induced in the protein folding process. The evidence further suggests that the resulting SEA heterodimers have evolved to dissociate as a result of mechanical rather than chemical forces. The physiological consequences of such properties and the possible relation to mucus layer regulation will be discussed.

References:

E. Wahlberg & T. Härd, *J. Am. Chem. Soc.* **128**, 7651-7660 (2006).
B. Macao, D.G.A. Johansson, G.C. Hansson & T. Härd
Nature Struct. Mol. Biol. **13**, 71-76 (2006).
V. Dincbas-Renqvist, C. Lendel, J. Dogan, E. Wahlberg & T. Härd
J. Am. Chem. Soc. **126**, 11220-11230 (2004).
E. Wahlberg *et al., PNAS* **100**, 3185-3190 (2003).

P-418

Photolysis studies on the binding between CO-cyt *c* mutant complexes and cyt *c* oxidase or cardiolipin

I. Husu, G. Silkstone, M. T. Wilson
Dept. of Biological Sciences, University of Essex, Colchester, U.K.

High rates of sub-millisecond electron transfers in cells preclude their study by mechanical mixing of reactants (e.g. stopped-flow). The use of light pulses instead, obvious with photosynthetic systems, can be extended to other pigmented proteins.
Our work relies on the high affinity of the ligand carbon monoxide (CO) for Fe^{2+} heme proteins with vacant sixth coordination position. Bound CO can be photo-cleaved with quantum yield ϕ, giving Fe^{2+} penta-coordinate hemes and lowering protein potential to electron donors' values. If CO does not rapidly rebind (recombine), photo-induced electron transfer can occur.
Eukaryotic cytochrome *c* (cyt *c*) transfers electrons from bc_1 complex to cyt *c* oxidase in the mitochondrial space. In native cyt *c* the heme iron is hexa-coordinated, with two internal axial ligands (His18, Met80), then unable to bind CO. So we produced some Met80 mutants of yeast iso-1-cyt *c* able to bind CO.
Met80Ala, Ser, Asp, Glu show small ϕ values due to fast *geminate* recombination after photolysis (CO unable to reach bulk solution from protein moiety). Met80Arg and Phe82 double mutants instead exhibit higher ϕ values because of more open and flexible conformations allowing better gas exchange: these may lead to successful laser flash induced electron transfer experiments.
CO recombination in mutants, measured by flash photolysis, led to kinetics influenced by structural properties. We analysed interactions of mutants with:
– cardiolipin, related to apoptosis where cyt *c* plays a key role;
– cyt *c* oxidase, to determine their binding constants.
In both cases results were obtained from φ changes and recombination rates and compared to those from other binding studies.

P-420

Optimisation of the enzyme nitroreductase for Gene-Directed Enzyme Prodrug Therapy

D. Jarrom[1], M. Jaberipour[2], P. R. Race[1], S. Daff[4], C. Guise[2], G. Burns[3], E. I. Hyde[1], S. A. White[1]
[1]School of Biosciences, [2]Institute for Cancer Studies, [3]School of Chemistry, The University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K., [4]School of Chemistry, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, U.K.

Gene-Directed Enzyme Prodrug Therapy (GDEPT) involves the introduction of a non-native enzyme into tumour tissue, which then converts an administered harmless prodrug to a cytotoxic drug, selectively killing tumour cells. One promising enzyme-prodrug combination is the *E.coli* enzyme nitroreductase (NfsB) with the prodrug CB1954. However, the wild-type enzyme exhibits poor kinetics, and therefore slow turnover, of CB1954. Site-directed mutagenesis followed by screening for improvements in CB1954 activity *in vivo* has produced several mutants that are more active than wild-type enzyme. This study compares the steady-state and stopped-flow kinetics of the most promising single, double and triple mutants with the wild-type enzyme, using CB1954 and other substrates. The most active mutant exhibits a 70-fold enhancement in specific activity with CB1954 compared to wild-type NfsB. Using nitrofurazone as substrate, little rate enhancement over wild-type is seen, showing that the mutations have selectively enhanced CB1954 activity. NfsB reduces CB1954 to one of two isomers, one of which is greatly more cytotoxic than the other. Analytical HPLC has shown that certain mutants favour the production of the more cytotoxic isomer, further enhancing the efficacy of the mutants.

Abstracts

– Protein-ligand interactions –

P-421

the adhesion stage of fertilization probed by sperm/egg adhesion force measurements.

A. Jegou[1], A. Ziyyat[2], C. Gourier[1], J.-P. Wolf[2]

[1]Laboratoire de physique Statistique, CNRS UMR8550, Paris, France, [2]Laboratoire de Biologie de la Reproduction, UFR SMBH Université Paris 13, Bobigny, France

Mammal fertilization is the union of an egg and a spermatozoon, a specific sequence of events that ends in the male and female pronuclei meeting to form a new being. After maturation of the gametes, the migration of sperm in the reproductive tract, a spermatozoon crosses the zona pellucida surrounding the oocyte to finally enabling the membranes of the two cells to contact.

The fusion of the gametes is a two-step process including attachment of two plasma membranes through cell-surface molecules and physical merger of their plasma membrane lipids.

So far, because of technical limitation, the molecular mechanisms involved in the adhesion and fusion stages could not be clearly distinguished.

We developed a new technique to study the adhesion stage of fertilization independently from fusion. It consists in directly measuring the adhesion force between two unique gametes through the adaptation of the Biomembrane Force Probe design by firmly binding the spermatozoon to the probe.

We will present typical curves showing the adhesion occurring between gametes. By tuning the contact time and maximum contact force we will show how to discriminate microvilli and microvilli-free regions of the oocyte membrane. Those results can be correlated with physiological fusion sites on the oocyte membrane of the spermatozoon.

P-422

Modulation of the channel activity of Vpu from HIV-1

P. Judge[1], G. Patargias[2], K. Varga[2], M. Triba[2], W. Fischer[3], A. Watts[2]

[1]Bionanotechnology IRC, Clarendon Laboratory, Department of Physics, Parks Road, OX1 3PU, Oxford, U.K., [2]Biomembrane Structure Unit, Department of Biochemistry, South Parks Road, OX1 3QU, Oxford, U.K., [3]Biophotonic Interdisciplinary Research Center, National Yang Ming University, 112 Taipei, Taiwan

Vpu is a viral ion channel protein encoded by HIV-1 to enhance the rate of virus particle release from an infected host cell by altering the electrochemical potentials across host lipid membranes. The 82 amino acid monomers have a single transmembrane domain, which oligomerises within the sub cellular membranes of the host forming pentameric bundles which enable ion flux. In this research, two strategies for inhibiting the activity of Vpu are under investigation as potential anti-viral therapies.

The inhibition of ion permeation by the amphipathic ligand HMA has been studied using channel recording of the transmembrane domain of Vpu reconstituted into black lipid membranes. The mechanism of inhibition has been probed by solid state NMR and molecular dynamics simulations.

A novel peptide inhibitor, based on the first transmembrane helix of the mammalian potassium channel TASK has been designed to bind to the monomeric form of Vpu, preventing oligomerisation and pore formation. Docking and molecular dynamics simulations have been used to optimise the amino acid sequence of the inhibitor peptide and to predict binding constants.

P-423

Effect of garlic extract on growth, medium pH and membrane potential in maize coleoptile segments

W. Karcz, M. Polak

University of Silesia, Faculty of Biology, Department of Plant Physiology, Katowice, Poland

Garlic (*Allium sativum L.*) exhibits a wide range of properties including antibacterial, antifungal and antioxidant activity. It is presently well established that the main active component of fresh aqueous garlic extract (GE) are alk(en)yl thiosulfinates, which are produced from alk(en)ylcysteine sulfoxides in the enzymatic reaction catalized by alliinase (alliin-lyase; E.C.4.4.1.4) after the grinding of garlic. Garlic extract contains eight different thiosulfinates, of which diallyl thiosulfinate (allicin) makes up 60-80%. Allicin undergoes thiol-disulphide exchange reactions and can react with SH-containing enzymes, causing their inhibition. Moreover, its antioxidant properties and ready membrane permeability are also regarded as the basis of its high biological activity. There have been no reports on the effect of GE on plant growth. The main objective of the present study was to determine the effects of auxin (IAA) and fusicoccin (FC) on growth, medium pH and membrane potential in maize coleoptile segments incubated in the presence of GE. Growth experiments were carried out in an apparatus, which allowed simultaneous measurements of elongation growth and pH of the incubation medium. Simultaneous measurements of growth and external medium pH indicated that GE inhibited IAA- and FC-induced growth and proton extrusion. Moreover, it was also found that GE caused membrane potential depolarization. A plausible interpretation for GE-induced changes in growth of maize coleoptile segments is that, at least in part, these changes are mediated via PM H^+-ATPase activity.

P-424

The effect of the actin binding proteins on the conformation of the ATP binding cleft on actin

R. Kardos[1], K. Pozsonyi[1], A. Vig[1], M. Nyitrai[1], E. Nevalainen[2], P. Lappalainen[2], G. Hild[1]

[1]University of Pécs, Faculty of Medicine, Department of Biophysics, Pécs, Szigeti str. 12, H-7624, Hungary, [2]Program in Cellular Biotechnology, Institute of Biotechnology, University of Helsinki, 00014 Helsinki, Finland

Actin is one of the most abundant proteins in all eukaryotic cells. The dynamics and organisation of the actin filaments in cells are regulated by a large amount of actin-binding proteins. Cofilin is a conserved regulator of cytoskeletal dynamics. It can promote the depolymerisation of actin filaments and inhibit the nucleotide exchange on actin monomers as well. Another central regulator of actin monomer pool is profilin, which facilitates the incorporation of actin monomers into the filament barbed end, and can promote the nucleotide exchange on actin monomers as well. We investigated the effect of cofilin and profilin on the structure of actin monomers around the ATP binding pocket. The fluorescence of the actin bound ε-ATP was quenched with a neutral quencher (acrylamide). The data were analysed with a modified form of the Stern-Volmer equation. With the help of this special form it is possible to separate the fluorescence signal coming from the actin bound and the free ε-ATP in the solution. The experiments revealed that in the presence of cofilin the accessibility of the bound ε-ATP decreased, indicating a closed and more compact ATP-binding pocket induced by the presence of cofilin. Contrary to this, in the presence of profilin the accessibility of the bound ε-ATP increased, indicating a more approachable protein matrix around the ATP-binding pocket.

Abstracts

– Protein-ligand interactions –

P-425

Atomic-scale visualization of iron-peroxide intermediates in superoxide reductase

G. Katona[1], P. Carpentier[1], V. Niviere[3], P. Amara[1], V. Adam[2], J. Ohana[1], N. Tsanov[1], D. Bourgeois[2]

[1]IBS, CNRS/CEA/UJF, Grenoble, France, [2]ESRF, Grenoble, France, [3]LCBM, iRTSV-CEA/CNRS/UJF, Grenoble, France

Iron-peroxide intermediates are involved in the catalytic cycle of many iron-containing enzymes. Such intermediates were also identified in superoxide reductase (SOR) a non-heme mononuclear iron-enzyme that neutralizes superoxide radicals. By diffusing hydrogen peroxide into SOR crystals, we trapped iron(III)-(hydro)peroxo species. The combination of X-ray diffraction data and non-resonant Raman spectra recorded *in crystallo* revealed "end-on" iron-(hydro)peroxo configurations [G. Katona et al., *Science,* in press]. *In crystallo* Raman spectroscopy was instrumental in this study. First, it allowed monitoring the influence of X-ray radiation on the trapped intermediates. Second, the ^{18}O isotopic shifts of the iron-peroxide specific vibration bands confirmed the direct involvement of hydrogen peroxide in the formation of the intermediates.

We found that the open SOR active site promotes the formation of transient hydrogen bond networks, which presumably assist the cleavage of the Fe-O bond in order to release the reaction product, hydrogen peroxide. Multiple observations of the SOR active sites in the asymmetric unit allowed us to propose a structural mechanism which directs the processing of reactive oxygen species without producing highly reactive iron (IV) oxo intermediates. Density functional theory studies provided further details on the protonation state of the intermediates.

O-426

Combining isothermal titration calorimetry, crystallography and computational approaches to understand protein-ligand interactions

G. Klebe

Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Germany

Binding affinity and selectivity prediction is an essential element of structure-based drug design. In virtual screening, docking methods are consulted to suggest novel leads, however, the ranking of putative hits according to their expected affinity remains the most crucial step in this procedure. Accordingly, understanding the driving forces for ligand binding is of high relevance to the whole field of drug design. Factorizing the free energy of binding into enthalpic and entropic portions will help to optimize a given ligand either towards selectivity or promiscuity of binding or to better cope with mutational differences of the target protein(s).

A combination of crystal structure analysis, isothermal titration calorimetry and molecular dynamics simulations has been applied to assess the binding properties of three initial virtual screening hits discovered for aldose reductase, a promising target to prevent diabetic long-term complications. The analysis provides insight into the thermodynamic binding profile of these ligands and defines further steps of lead optimization. Prior to a reliable factorization into enthalpic and entropic binding contributions a detailed analysis of changes of protonation states had to be performed. Apart from pKa calculations, mutagenesis and crystal structure analysis provide a detailed picture on the proton transfer steps overlaid with the actual binding process.

P-427

Ligand dynamics and early signaling in the heme-based oxygen sensor proteins Dos and FixL

U. Liebl, T. Yamashita, L. Bouzhir-Sima, M. H. Vos

LOB, Ecole Polytechnique, Palaiseau, France

FixL and Dos are bacterial oxygen sensor proteins, in which binding/release of O_2 to/from a heme-cofactor induces changes in the activity of an associated enzymatic domain. While the structures of the heme-domains FixLH and DosH are generally similar, their heme coordination is markedly different: in FixLH the unliganded heme is 5-coordinated, like in myoglobin, whereas in DosH O_2 replaces an internal ligand (Met 95). Heme-ligand photodissociation can be used to trigger 'switching' of the sensor and identify intermediates in the intra-protein signalling pathway. Ultrafast absorption experiments indicate that unusual heme-ligand interactions are maintained after dissociation, with the heme pocket acting as efficient oxygen trap (1,2). Using wild type and site-specific mutant proteins, we observed substantial changes in early heme-ligand dynamic motions. Their functional implications will be discussed. Time-resolved resonance Raman spectroscopy measurements revealed that surprisingly no sizeable heme doming is observed in the FixLH oxycomplex, likely due to highly efficient ultrafast O_2 rebinding to the heme on the fs time scale (3). Recent temperature-dependence measurements on DosH indicate that geminate ligand rebinding processes are barrierless, whereas ligand escape is not.

1. Liebl U, Bouzhir-Sima L, Négrerie M, Martin JL, Vos MH (2002) *PNAS* **99,** 12771-12776.

2. Jasaitis A, Hola K, Bouzhir-Sima L, Lambry JC, Balland V, Vos MH, Liebl U (2006) *Biochemistry* **45**, 6018-6026.

3. Kruglik SG, Jasaitis A, Hola K, Liebl U, Martin JL, Vos MH (2007) *PNAS* **104,** *in press*.

P-428

Backbone structure of neuropeptide bradykinin bound to the human GPCR bradykinin-2

J. J. Lopez[1], A. Shukla[2], C. Reinhart[2], H. Schwalbe[3], H. Michel[2], C. Glaubitz[1]

[1]Center for Biomolecular Magnetic Resonance & Institute for Biophysical Chemistry, Goethe University Frankfurt, Germany, [2]Max Planck Institute for Biophysics, Frankfurt, Germany, [3]Center for Biomolecular Magnetic Resonance & Institute for Organic Chemistry, Goethe University Frankfurt, Germany

The backbone structure of bradykinin, a high affinity, linear nonapeptide hormone (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9), was determined using solid-state NMR spectroscopy whilst bond to the human B2 G-protein coupled receptor. We have used ^{13}C and ^{15}N secondary chemical shifts to obtain Φ/Ψ dihedral angle constraints which were used for a molecular dynamics based structure calculation (RMSD 0.5Å). Experiments were carried out on DDM solubilised receptor at low temperatures using ^{13}C double quantum filtered MAS NMR spectroscopy. Our data reveal an overall twisted S-shape of the ligand with an N-terminal β-turn formed by Ser^6-Pro^7-Phe^8-Arg^9. Pro^2, Pro^3 and Pro^7 were found in their *trans* conformation. The receptor bound structure of bradykinin will be compared with the peptide structure in solution and in a membrane mimicking environment. It will be discussed in the context of known biochemical data.

As bovine rhodopsin is still the only GPCR with known 3D structure, the structure determination of bound hormones is potentially of high pharmacological interest for the rational drug design of synthetic agonists and antagonists.

Abstracts

– Protein-ligand interactions –

P-429

The structure of neurotransmitters and amino acids in aqueous solution

S. E. McLain[1], A. K. Soper[3], J. W. Taylor[3], A. Watts[2]
[1]Oak Ridge National Laboratory, U.S.A., [2]Departement of Biochemistry, University of Oxford, U.K., [3]ISIS Facility, Rutherford Appleton Laboratory, U.K.

The structure of biological molecules - namely neurotransmitters and amino acids - in aqueous solutions has been investigated using **n**eutron **d**iffraction augmented with **i**sotopic **s**ubstitution (NDIS). NDIS is a well-proven technique for the determining the structure of hydrogen containing molecules in aqueous solution. The data collected were subsequently modeled using the disordered materials program **E**mpirical **P**otential **s**tructural **r**efinement (EPSR) which provides a molecular structural model of the systems studied that is not only consistent with the measured diffraction data but also physically reasonable. Using this combination of NDIS and EPSR, site-specific information concerning the intermolecular interactions between the amino acid solute and the surrounding water solvent as well as the solvent-solvent interaction on a local level (1-10 Å scale) are elucidated.

P-430

MHC complex stability: Shaping antigenic features through long-range electrostatic interactions

D. Narzi[1], K. Winkler[2], J. Saidowsky[2], R. Misselwitz[3], A. Ziegler[3], R. A. Böckmann[1], U. Alexiev[2]
[1]Theoretical & Computational Membrane Biology, Center for Bioinformatics Saar, Saarland University, Germany, [2]Physics Department, Freie Universität Berlin, Germany, [3]Institut für Immungenetik, Charité Universitätsmedizin Berlin, Humboldt-Universität zu Berlin, Germany

A single difference between HLA-B*2705 and HLA-B*2709 (Asp116His) is responsible for the emergence of distinct T cell repertoires in individuals harbouring either of these two MHC alleles and is expected to be coupled to their differential association to the autoimmune disease ankylosing spondylitis. By using fluorescence depolarization and pKa calculations we investigated to what extent short- and long-range electrostatic interactions contribute to shape antigenic differences between HLA-B*2705/09 molecules complexed with viral, self and non-natural peptides. In addition to the established main anchor of peptides binding to HLA-B27 (pArg2) and the secondary anchors at the peptide termini, two further determinants contribute to stable peptide accommodation: (1) Complexes with pLMP2 and pVIPR are stabilized by about 5 kJ/mol in HLA-B*2705 with respect to HLA-B*2709, through the interaction of pArg5 with Asp116. (2) Long-range electrostatic interactions are shown to strongly influence the pKa of the heavy chain residues Glu45 and Glu63 in the B-pocket, which are key residues for the interaction with pArg2. Protonation of Glu45/63 results in a reduction of the interaction energy between pArg2 in pLMP2 and the respective subtype of up to 20 kJ/mol. Thus, a peptide-dependent receptor protonation modulates complex stability and antigenic features of the respective HLA-B27 subtypes.

P-431

Quantification of Melanoma Cell Adhesion to the Protein von Willebrand Factor

T. M. Neumaier[1], A. Wixforht[2], K. Sritharan[2], T. Franke[2], S. W. Schneider[3], M. F. Schneider[2]
[1]GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany, [2]Lehrstuhl für Experimentalphysik I, Biophysik, Universität Augsburg, Universitätstraße 1, 86135 Augsburg, Germany, [3]Institut für Dermatologie, Universität Münster, Von-Esmarch-Straße 58, 48149 Münster, Germany

The adhesion of carcinoma cells to the extracellular matrix within the blood vessel is an important step in cancer cell invasion. The protein *von Willebrand factor* (vWF) plays a key role in platelet adhesion during the blood clotting process and may facilitate the adhesion of melanoma cells when it undergoes metastases. By using the micropipette aspiration technique which enables us to manipulate a single melanoma cell onto glass slides biofunctionalised with the vWF, we were able to quantify the adhesion energy between the melanoma cell and the vWF. The use of surface acoustic wave induced streaming allowed to mimic shear rates similar to our micro-circulatory system. We report that once a critical shear is applied vWF induced adhesion is increased by 25%. The shear rate applied necessary coincides with earlier studies where vWF was found to undergo a coiled stretched transition under high shear rate conditions. The importance for the VWF mediated metastasis is discussed.

P-432

P-glycoprotein models based on Sav1866 and MalK: predicted transition from ATP-bound to apo state

M. L. O'Mara, D. P. Tieleman
University of Calgary, Calgary, Canada

The ATP Binding Cassette (ABC) multi-drug transporter P-glycoprotein (P-gp) prevents the cellular uptake of toxins by using the energy derived from ATP hydrolysis to actively transport a wide range of structurally unrelated compounds out of the cell including chemotherapeutic drugs, antibiotics and steroid hormones. Continuous administration of therapeutic drugs can lead to the overexpression of P-gp, resulting in multidrug resistance in cancers and HIV. At present, the precise structural details of the P-glycoprotein drug binding sites and the mechanism of substrate translocation are unknown. Crystallisation of the bacterial ABC transporter, MsbA, allowed the development of a structural framework for P-glycoprotein. The recent crystallisation of a second bacterial multidrug ABC transporter, Sav1866, revealed the topology of the two transporters is incompatible, leading to the withdrawal of MsbA and prompting a reinterpretation of many P-gp results. Here we exploit the biochemical and sequence similarity between Sav1866 and P-glycoprotein to develop a homology model of P-glycoprotein representing an ATP-bound state. The model captures the major features of the low-resolution EM structure and is consistent with cysteine mutagenesis studies. Using insights from the MalK crystal structures, we model two nucleotide-free conformations. Conformational changes are characterised by pincering rigid-body rotations of the nucleotide-binding domains, inducing transmembrane domain reorganisations which correspond to the two lowest frequency normal modes of the protein. These conformations may characterise major steps in the nucleotide catalytic cycle.

Abstracts

– Protein-ligand interactions –

P-433

Structure, function and mechanism of action of the FtsZ protein in prokaryotic cell division

R. Pacheco-Gomez, A. Rodger, D. I. Roper
University of Warwick, UK

Biological cells contain the machinery of life. Cell morphology and the correct division of cells during meiosis are controlled by the cytoskeleton – a collection of fibrous proteins. Our understanding of the conformations of the proteins that make up these fibres is limited due to the fact that fibrous proteins are both insoluble and heterogeneous in length.

We have been working on the bacterial cytoskeleton protein FtsZ. FtsZ, a functional homologue of tubulin, is a polymer-forming GTPase. It also plays a critical role in cell division of prokaryotic cells. FtsZ assembles into a cytokinetic ring at the equator of the cell (the Z-ring) on the inner surface of the cytoplasmatic membrane that carries out cell decision in prokaryotes. The Z-ring recruits at least ten other proteins, all of which play a part in allowing cell division to proceed as the Z-ring reduces in diameter until cytokinesis is complete. Elucidating the mechanism(s) by which the Z-ring drives cell division is therefore critical to our understanding of cytokinesis in the majority of prokaryotes and some eukaryotic organelles.

Some techniques that have traditionally been used to examine and predict possible interactions and conformational changes in FtsZ include Circular Dichroism Spectroscopy (CD), indirect biophysical measurements and the direct visualization of the polymers using Electron Microscopy (EM). Here, we present the use of a novel technique, Linear Dichroism Spectroscopy (LD), used for the first time on the analysis of FtsZ's dynamic behaviour and protofilament interaction.

P-434

Creation of cellular adhesion by anchoring proteins to membranes using the diphtheria toxin T domain

A. Perier[1], C. Gourier[2], S. Pichard[1], J. Husson[2], E. Lajeunesse[1], A. Babon[1], A. Menez[1], D. Gillet[1]
[1]CEA, DSV, Département d´Ingénierie et d´Etudes des Protéines, CEA-Saclay, 91191 Gif-sur-Yvette, France, [2]LPS-ENS, CNRS, Universités Paris 6, Paris 7, 24 rue Lhomond, 75005 Paris, France

Proteins expressed at the cell membrane control cell signaling, recognition, binding and stimulation processes. Cell surface engineering is therefore of great potential in the development of cell therapies. To modify membrane protein expression, cells are generally manipulated by transfection (introduction of genes into the cells). Here, we propose an alternative way of bringing new proteins to the cell surface without any transfer of genetic material. The method is based on the use of a membrane anchor derived from the diphtheria toxin translocation (T) domain. This soluble T-protein binds to cell membranes after incubation at acidic pH. It can be used to attach to cell surfaces any soluble protein fused to its N- or C-terminal end. To test the potential of such a method, we have considered a fusion protein (FLT) in which the FL homodimeric cytokine is fused to the N-terminus of the T domain. We have shown that FLT, anchored to the surface of tumor cells is still functional, and can trigger the activation of cells carrying the Flt3 receptor. With a dual pipette assay that allow to quantify intercellular adhesion in terms of mechanical forces at the cellular level we demonstrated that FLT-anchoring cells bind to Flt3-expressing cells and proved that this adhesion is specifically due to FLT/Flt3 interaction. This study shows that the anchoring of protein ligands to the surface of cells is an efficient way to control cell surfaces and therefore cell interactions.

P-435

Label free analysis of protein interactions by time resolved UV fluorescence spectroscopy

S. Peters[1], W. Fritzsche[1], P. Grigaravicius[2], K. O. Greulich[2], H.-M. Striebel[2], P. Schellenberg[1]
[1]Institute of Photonic Technology, Jena, Germany, [2]Leibniz – Institute for Age Research – Fritz Lipmann Institute, Jena, Germany

The label-free detection of protein-protein and protein-ligand interactions presents a challenge, which has yet been met only by a few methods such as mass spectrometry, imaging ellipsometry and surface plasmon resonance. These techniques are favourably complemented by a new approach based on the fluorescence decay pattern analysis of the proteins' intrinsic fluorescence in the UV-range of the spectrum. By this method, the aromatic amino acids TRP and TYR serve as internal probes to detect alterations of their environment upon coupling to a binding partner, which is reflected in a change of their fluorescence lifetime. Notably, binding of small ligands to proteins can be probed, that may evade detection by surface plasmon resonance or ellipsometry, which are only sensitive to the attachment of medium and large binding partners.

The feasibility of label-free fluorescence lifetime analysis of proteins on microarrays as well as in solution has been shown for several types of binding partners, such as protein-protein, protein-oligonucleotide aptamers and protein-ligand pairs.

The protein chip analysis was automated by spacially scanning the chip surface. The decay data were collected by time correlated single photon counting thereby getting a fluorescence lifetime image. By this means, label free highly parallelized protein interaction analysis becomes available.

Lit.: H.M. Striebel et al., Proteomics 4, (2004), 1703–1711.

P-436

Ezrin interaction with PIP2-containing vesicles

C. Picart[1], G. Blin[1], E. Margeat[2], C. A. Royer[2], C. Roy[1]
[1]Univ. Montp 2, CNRS UMR 5235, Montpellier, France, [2]Centre de Biochimie Structurale, Montpellier, France

The plasma membrane-cytoskeleton interface is a dynamic structure, participating in a variety of cellular events including cell shape and motility. Among the proteins involved in the direct linkage between components of the cytoskeleton and the plasma membrane is the ezrin/radixin/moesin (ERM) family. The FERM domain in their N-terminus contains a PtdIns(4,5) binding site responsible for membrane-binding. In this work, we quantified the interaction of ezrin with large unilamellar vesicles (LUVs) containing PIP_2. We synthesized human recombinant ezrin bearing a cysteine residue at its C-terminus for subsequent grafting with Alexa488 maleimide. The affinity constant between ezrin and LUVs was determined by co-sedimentation assays and fluorescence correlation spectroscopy. The percentage of ezrin bound to LUVs containing 5% PIP_2 or 20% PS was estimated after centrifugation either after electrophoresis for WT ezrin or by spectrofluorimetry for A488-ezrin. For FCS measurements, the fraction of ezrin bound was determined by fitting the autocorrelation curves with two populations. The association constant was found to be $\sim$5 μM for PIP_2-LUVs and 20 to 70 lower for PS-LUVs. These results demonstrate, as well, that the interaction between ezrin and PIP_2-LUVs is not cooperative. Finally, by varying the ezrin concentration in presence of LUVs at a fixed lipid concentration, we found that ezrin FERM domain (area of $\sim$ 30 nm^2) binding to a single PIP_2 can block access to neighboring PIP_2 molecules and thus contributes to lower the accessible PIP_2 concentration. In addition, no evidence exists for a clustering of PIP_2 either in the absence of ezrin or induced by ezrin addition.

Abstracts

– Protein-ligand interactions –

P-437

A highly energetic intermediate in SNAREpin assembly

F. Pincet[1], F. Li[1], E. Perez[1], W. Eng[2], T. Melia[2], J. Rothman[2], D. Tareste[2]

[1]Laboratoire de Physique Statistique, Ecole Normale Supérieure, 24 rue Lhomond, 75005 Paris, France, [2]Department of Physiology and Cellular Biophysics, Columbia University, 1150 Saint-Nicholas avenue, New York, NY 10032, USA

Membrane fusion occurs when SNAREpins fold up between lipid bilayers. How much force is generated and how it is coupled to the apposing membranes is still a mystery. We have utilized the Surface Forces Apparatus to determine the energetics and dynamics of SNAREpins formation and characterize the different intermediate structures sampled by cognate SNAREs in the course of their assembly. The force *versus* distance profiles of assembling SNAREpins reveal a discrete intermediate corresponding to partially zippered complexes. These partially assembled SNAREpins are irreversible on biological timescales and, as such, can maintain a close contact between membranes during the molecular orchestration of the fusion machinery. Importantly, the energy stabilizing a single SNAREpin in this intermediate stage (35 k_BT) corresponds closely with the energy needed to fuse outer but not inner leaflets (hemifusion) of pure lipid bilayers. The fully zippered SNAREpin is of greater energy and has the potential to rearrange lipids during membrane fusion.

P-438

Effect of galantamine on muscular nicotinic receptors desensitization – a patch-clamp study

A. Popescu[1], A. Bicho[2], T. Moura[2], C. Ganea[1]

[1]Dept. of Biophysics, UMF "Carol Davila", Bucharest, Romania, [2]REQUIMTE, Dep. Quimica, FCT, Universidade Nova de Lisboa, Caparica, Portugal

Galantamine is an acetylcholine-esterase inhibitor, which has also been reported to act by allosterically potentiating the binding of acetylcholine (ACh) to the nicotinic receptor (Santos 2002, Akk 2005). Here we report, for the first time to our knowledge, an effect of galantamine on the desensitization of the muscular nicotinic receptor. These receptors are naturally expressed by the TE671LH cell line, which was used here for the whole-cell patch-clamp characterization of ACh+galantamine elicited ionic currents, under voltage-clamp conditions. In order to investigate the receptor short-time desensitization, ACh and/or ACh+galantamine have been applied with a fast perfusion system in trains of pulses at 5 seconds intervals, at different concentrations of ACh (10-50 uM) and galantamine (0.1-5 uM). Receptor desensitization has been evaluated from the decrease of the peak-current amplitude in a train of elicited responses. Different responses can be obtained in the presence of ACh and galantamine, depending on their relative concentrations: (i) a less significant decrease of the amplitude of the peak currents in a train of elicited responses as compared to the application of ACh alone (control), (ii) no alteration of the peak currents, or (iii) an increase of nicotinic currents, after initial desensitization. Our data suggest that the pattern of response is less dependent on the absolute values of ACh and galantamine concentration and influenced more by the balance between the two drugs and the number of available nicotinic receptors.

P-439

An original approach to FRAPP experiments: Study of interactions between membrane proteins

M. Reffay[1], Y. Gambin[1], H. Benabdelhak[2], A. Ducruix[2], W. Urbach[1]

[1]Laboratoire de Physique Statistique de l´ Ecole Normale Supérieure, UMR 8550 (CNRS-Université Paris 6), 24 rue Lhomond, 75005 Paris, France, [2]Laboratoire de R.M.N. et Cristallographie Biologiques, UMR 8015 (CNRS-Université Paris 5), 4 avenue de l´Observatoire, 75006 Paris, France

Membrane protein interactions are involved in a large array of biological processes: trafficking through export, antibiotic resistance such as efflux pump, ... However interactions between membrane-bound proteins are not easy to study with the numerous techniques used with soluble proteins. To address this issue, we develop a novel approach based on diffusion measurements.

P. aeruginosa (a Gram-negative bacteria) displays a multidrug resistance mechanism to export toxins out of the cell. The pump consists of the inner membrane-spanning proton-antibiotics antiporter protein MexB, the outer membrane associated channel OprM and the membrane fusion protein MexA.

To understand the way the proteins interact we focus on the interaction of MexA, with the outer membrane protein. Proteins are incorporated in model bilayers whose separation is tunable at will. We show that, thanks to a screening of the approach conditions between the two selected proteins, FRAPP technique allows to detect the interaction as well as to determine the configuration and the stoichiometry of the complex, which constitute a major challenge. It allows also the measure of unbinding times between MexA and OprM and the estimation of their interaction energy.

P-440

Peptide-protein recognition using static (de)quenching methodologies

M. M. Ribeiro, H. G. Franquelim, M. A. Castanho

Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande C8, 1749-016 Lisboa, Portugal

Protein-protein interactions, as well as peptide-peptide and peptide-protein interactions are fields of study of growing importance as molecular-level detail is avidly pursued in drug design, metabolic regulation and molecular dynamics, among other classes of studies. However, experimental tecnhiques and methodologies to detect and quantify such interactions are not abundant. A realiable, fast and inexpensive alternative methodology is revisited in this work.

Considering the interaction of two molecules, at least one of them being fluorescent, either intrinsically (e.g Trp residues) or by grafting a specific probe, changes in their aggregation state may be reported. As long as the fluorophore is sensitive to local changes in polarity, conformation and/or exposure to the solvent, interaction will probably lead to modifications in fluorescence intensity resulting in a decrease ("quenching") or enhancement ("dequenching"). Although the presented methodology is based on static quenching methodologies, the concept is extended from quenching to any kind of interference with fluorophore.

Equations for data analysis are shown and their aplications are illustrated by calculating the binding constant for several data sets.

Abstracts

– Protein-ligand interactions –

P-441

Light-induced metarhodopsin II/metarhodopsin III conversion investigated by time-resolved FTIR

E. Ritter, M. Elgeti, K. P. Hofmann, F. J. Bartl
Institute for Medical Physics and Biophysics, Charité, Universitätsmedizin Berlin, Germany

Vertebrate rhodopsin shares with other retinal proteins the 11-cis-retinal chromophore and the light-induced cis/trans isomerization triggering its activation. However, only in rhodopsin the retinylidene Schiff base bond to the apoprotein is eventually hydrolyzed, making a complex regeneration pathway necessary. Metabolic regeneration cannot be short-cut by reversed trans/cis isomerization. Instead, light absorption in the active metarhodopsin (Meta) II causes anti/syn isomerization of the retinylidene linkage. A new deactivating pathway is thereby triggered, which ends in the Meta III "retinal storage" product. Using time-resolved Fourier transform infrared spectroscopy, we show that identified steps of receptor activation, including Schiff base deprotonation and protein structural changes are all reversed. However, Schiff base reprotonation is much faster than the activating deprotonation, whereas protein structural changes are slower. The final proton release occurs with a pK similar to the pK at which the isolated opsin apoprotein becomes active. A forced deprotonation, equivalent to the forced protonation in the activating pathway is not observed. This explains properties of the final Meta III product, which displays much higher residual activity and is less stable than rhodopsin arising from regeneration with 11-cis-retinal. We propose that the anti/syn conversion can induce a fast reorientation and distance change of the Schiff base but fails to build up the full set of dark ground state constraints, presumably involving the Glu(134)/Arg(135) cluster.

P-442

The proteins role in regulation of interaction between axon and Shwann cell in the myelinated nerve fibre

N. N. Rodionova, N. A. Brazhe, G. V. Maksimov
Biological Faculty, Moscow State University, Moscow, Russia

The character of the axon-Shwann cell interaction in the myelin nerve fibre is determined by some proteins: nodal ($Na_v1.6$), paranodal (contactin, Caspr, NF 155, NF 186) and juxtaparanodal ($K_v1.1$, $K_v1.2$, Caspr-2) proteins. We study the effect of modification free SH-groups level of superficial proteins (pHMB affect), mild proteolysis of the superficial nerve proteins (pronase Å) and sorption on the nerve alien protein (OspA) on the axon-Shwann cell interactions. All these agents lead to the decrease in the action potential amplitude. The velocity of the conducting of action potential increases under the nerve proteins proteolysis, doesn't change during binding of free SH-groups of the superficial nerve proteins and decreases during the sorption OspA on the nerve surface. Changes of the nerve electrophysiological features may be related to the activity of Na^+,K^+-ATPase, Na^+-channels and K^+-channels and to the structure and properties of the myelin. We showed the decrease in the axolemma microviscosity (AM) during binding of free SH-groups and sorbtion of OspA, and the increase in AM under the nerve proteins proteolysis. We have shown, that sorption of OspA on the nerve surface leads to the decrease in AM accompanied by the redistribution of the membrane-bound Ca^{2+} ($Ca^{2+}{}_{mb}$) in the myelin and axolemma. The decrease in AM, caused by binding of free SH-groups of superficial nerve proteins, doesn't related to the change of $Ca^{2+}{}_{mb}$ level. We revealed changes of regular fluctuations of the local refractive index in paranodal and juxtaparanodal nerve areas under the proteolysis. The role of proteins in the regulation of axon-glial interactions and myelin structure is discussed.

P-443

Effect of cosolvents and water on protein binding, folding and stability

S. Shimizu
York Structural Biology Lab., Department of Chemistry, University of York, U.K.

There are various cosolvent molecules around the proteins affecting interaction, folding and stability. Sugars and polyols are used to enhance binding and crystallisation, and protect proteins from denaturation. Urea and guanidine destabilize proteins. Salts have varying degree of effects on protein stability (Hofmeister effect). In addition, crowding agents are known to enhance the binding as well as stabilising the protein. How can we understand the effect of such cosolvents on interaction equilibria and stability? We have developed a rigorous statistical thermodynamic theory (based upon the Kirkwood-Buff theory of solution) which can answer such questions at a molecular level. This theory can calculate the numbers of water and cosolvent molecules that are taken up or released upon reaction, based upon experimental data: high pressure measurement (how the equilibrium depend upon hydrostatic pressure) and the cosolvent concentration-dependence of equilibrium. Based upon this theory, we have clarified that (i) the difference between denaturant and stabiliser is the degree of accumulation/exclusion of the cosolvents on protein surface, (ii) molecular crowding is based upon the exclusion of cosolvents from protein surface, not the change of protein hydration, (iii) Hofmeister effect arises due to the salt-protein interaction, not due to the salt-induced changes in protein hydration. I shall also discuss how the different degrees of protein-cosolvent interactions arise, from a molecular basis.

P-444

X-ray structure and characterisation of a hydroxynitrile lyase from *Adenia racemosa*

N. Staunig[1], W. Höffken[2], C. Kratky[1], K. Gruber[1]
[1]Department of Chemistry-Structural Biology, Karl-Franzens-University Graz, Heinrichstrasse 28, A-8010 Graz, Austria, [2]BASF-AG, GVC/C - A 30, 67056 Ludwigshafen, Germany

Hydroxynitrile lyases (HNLs) are plant enzymes that catalyse the cleavage of cyanohydrins into hydrocyanic acid and the respective aldehydes or ketones. HNLs possess a high potential for industrial applications, because they also catalyse the inverse of the natural reaction *i.e.* the enantioselective synthesis of cyanohydrins. The (S)-HNL from *Adenia racemosa* (*Ar*HNL) is a glycosylated protein with a molecular mass of 12.4 kDa. It is homologous (with 31% sequence identity and 60% similarity) to Boiling Stable Protein SP1 (PDB-entry 1TRO).*Ar*HNL was successfully crystallised by the sitting–drop technique at room temperature with sodium acetate, trifluoroethanol and PEG-6000 as precipitating agents. The thus obtained monoclinic crystals belong to the space group *C2* with cell dimensions a=138.0 Å, b=52.3 Å and c=87.0 Å, b=126.70°. The structure was solved by molecular replacement and the refinement to a crystallographic resolution of 1.3 Å is still in progress. The current R values are: R= 18.6% and R_{free}= 21.8%.In the crystal, *Ar*HNL forms a homo-dimer, with each monomer consisting of a four-stranded anti-parallel β-sheet packed against 3 α-helices. The main contact between the monomers occurs via the β-sheets .Soaking experiments are in progress in order to elucidate the crystal structure of the enzyme substrate complex. In addition biophysical, enzyme kinetic and mutagenesis studies are planed.

Abstracts

– Protein-ligand interactions –

P-445

A structural study of receptor bound neurotensin by solid-state NMR

S. Tapaneeyakorn, K. Varga, P. J. Harding, H. Attrill, A. Watts
Biomembrane Structure Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

The tridecapeptide, neurotensin (NT), acts as a neurotransmitter in the central nervous system (CNS) and peripherally in the gastrointestinal tract as a local hormone. Its receptor, NTS1, belongs to the G protein-coupled receptor family and is a potential target for the treatment of pain, eating disorders, stress, schizophrenia, Parkinson's disease, Alzheimer's disease and cancer. The six C-terminal amino acids (8-13) are sufficient for binding to this receptor and eliciting the major pharmacological effects of the peptide. The aims of this study are to examine the conformation of the hexapeptide free and bound to its receptor using solid-state NMR, and to characterize further this interaction using other biophysical tools. ^{15}N-Pro, ^{13}C-Tyr, ^{13}C-Ile, and ^{13}C-Leu enriched $NT_{(8-13)}$ was produced by solid phase peptide synthesis and purified by reversed phase HPLC. ^{13}C cross-polarization magic-angle spinning (CP-MAS) NMR was used to determine the assignment for this peptide. To investigate the NT-NTS1 interaction, NTS1 was expressed in *E. coli* and purified. Functional ligand binding has been demonstrated using surface plasmon resonance (SPR). Furthermore, to facilitate solid-state NMR studies, NTS1 has been reconstituted into a model membrane system. ^{13}C REDOR and rotational resonance NMR is being employed to obtain structural and orientational data from the peptide bound to its functional receptor in a model membrane system.

P-446

Stability of nickel coordination compounds and their interactions with biological molecules

K. Tvetinskaya[1], A. M. Polyanichko[2]
[1]Faculty of Chemistry, St. Petersburg State University, Russia, [2]Faculty of Physics, St. Petersburg State University, Russia

Coordination complexes of nickel are known to have a great biological importance. In this work, several classes of the nickel complexes were compared in terms of their stability upon binding various ligands. The nature of the chemical bonds formed in the complexes with biological molecules is discussed. Several factors determining the configuration of the bonds within the complexes and distortions of electronic structure were revealed. The macro-synthesis of hexamine-Ni(II) was performed and the complexes with different type of symmetry were studied. The possibility to use complexes of nickel as express-indicators for different types of ligands was proven experimentally. In this study we made an attempt to classify the modes of interaction of coordination compounds of nickel with other ligands. Special attention was paid to the interaction with biological macromolecules, such as amino acids and DNA. We have shown, that the difference in binding between Ni^{2+} and Ni^{3+} can be attributed to the difference electronic structure of their complexes and their symmetry in particular.

P-447

Electrostatic potential of aminoacyl-tRNA synthetase navigates tRNA towards its binding region

D. Tworowski[1], A. Feldman-Salit[2], M. G. Safro[3]
[1]Weizmann Inst. of Science, Rehovot, Israel, [2]EML Research, Heidelberg, Germany, [3]Weizmann Inst. of Science, Rehovot, Israel

Unlike many other families of enzymes which catalyze the same overall reaction, aminoacyl-tRNA synthetases (aaRSs) are extremely heterogeneous in terms of primary sequence and subunit organization. For the most part aaRSs are negatively charged at physiological conditions, as are tRNA substrates. What are the driving forces that ensure an attraction between like-charged macromolecules? The diffusional association of aaRS and tRNA was found to be governed by long-range electrostatic interactions when homogenous negative potential of tRNA fits to the patches of positive potential produced by aaRS: one patch for each tRNA substrate molecule. Considering aaRS as a molecule with anisotropic reactivity and based on the continuum electrostatics and Smoluchowski's theory, the reaction conditions for tRNA-aaRS diffusional encounters were formulated. As may be inferred from multiple sequence alignments, concentration of the invariant charged residues in structural domains doesn't correlate with contribution of the domains to formation of the EP at long distances. The domains, categorized as enzymatically relevant, appeared to be nonessential for field sculpturing. Subdividing the aaRS's charged residues into native, conservative and non-conservative subsets we evaluated the contribution of each group to long-range EP. Surprisingly, the EP landscapes generated by native and non-conservative subsets are fairly similar, thus suggesting the non-conservative subset being specifically developed for efficient tRNA attraction.

P-448

Distinct mode of binding of nucleosomal core and linker histones to nucleoplasmin, a nuclear chaperone

M. A. Urbaneja, I. Arregi, J. Falces, S. Bañuelos, A. Muga, S. G. Taneva
Unidad de Biofísica (CSIC/UPV-EHU), Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, Spain

In *Xenopus laevis* eggs, nucleoplasmin (NP), a histone chaperone, acts as a reservoir for histones H2A and H2B and can displace sperm nuclear basic proteins and linker histones from the chromatin fiber of sperm and quiescent somatic nuclei, respectively. It has been proposed that NP mediates the dynamic exchange of histones during the expression of certain genes and assists the assembly of nucleosomes by modulating the interaction between histones and DNA.

To carry its biological activity NP needs to be hyperphosphorylated, with phosphate groups located in both of its domains, the "core" responsible for the pentameric nature of the protein and its high stability, and the "tail" that traditionally is related to its binding properties to basic proteins. To assign the contribution of phosphorylation of each NP domain to the binding to core and linker histone proteins, and due to the difficulty to obtain NP homogenously phosphorylated, we have generated NP substitution mutants where Asp mimics phosphorylatable residues. We have accomplished a comparative thermodynamic and spectroscopic study of the binding of the functionally distinct nucleosomal core H2A/H2B and linker H5 histones from chicken erythrocytes to different NP variants. Data show that (i) the mode and thermodynamics of NP binding to the core histone dimer H2A/H2B differs from that to the linker histone H5, and (ii) mutations in NP core and tail, mimicking phosphorylation, have a strong effect on the binding energetics.

Abstracts

– Protein-ligand interactions –

P-449

Biophysical approaches in dentistry

V. Vetterl[1], J. Vaněk[2], R. Silvennoinen[3], S. Hasoň[1], L. Strašák[1], P. Černochová[2], P. Prachár[2], S. Bartáková[2], E. Jančářová[4], S. Matalová[4]
[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno. Czech Republic, [2]Stomatological Research Center, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [3]Univerity of Joensuu, Department of Physics and Mathematics, Joensuu, Finland, [4]Faculty of Chemistry, Technical University, Brno, Czech Republic

We measured the growth of yeasts Saccharomyces cerevisiae on mercury electrode. We use measurement of differential capacitance, time dependence and impedance spectroscopy. We were able to control growth of microorganisms in broth on solid electrode. We detected the forming of biofilm on mercury surface and we were able to detemine the concentration of microorganisms in broth.Titanium is frequently used as a biomaterial for hard tissue replacement, such as dental and orthopaedic implants. The application of diffractive optical element (DOE) for the investigation of adsorption of biopolymers (human blood plasma proteins and nucleic acids) on the titanium surface was studied. The adhesion of model microorganisms Streptococcus mitis and Streptococcus salivarius present in oral plaque to the polymer component of dental restoratives was studied. The composition of dental restoratives was varied, thus influencing their hydrophobicity. Resin composites with antibacterial activity may be useful to decrease the frequency of secondary caries. This project was supported by the grant project No.1M0528 of the Ministry of Education and Sport of the Czech Republic.

P-450

NMR study of the interaction between nociceptin and the extracellular loop 2 of its receptor

B. Vincent, L. Mouledous, B. Bes, C. Topham, H. Mazarguil, J.-C. Meunier, A. Milon, P. Demange
IPBS, UPS-CNRS, Toulouse, France

The nociceptin (NOP) receptor is a G-Protein Coupling Receptor (GPCR) involved in pain regulation, stress, and drug addiction. The NOP receptor shares high sequence homology with the μ-, δ- and κ-opioid receptors and, similar to the κ-opioid receptor, possesses a highly acidic second extracellular loop (ecl2). Likewise, NOP, the natural agonist of the NOP receptor, is a highly basic heptadecapeptide that resembles dynorphin A, the natural agonist of the κ-opioid receptor. Both the NOP receptor ecl2 and NOP's basic core have been shown to be of primary importance for biological activity. In order to characterize the interaction between NOP and ecl2 we used a peptidic fragment approach. Structural and dynamic studies were made by NMR spectroscopy. A synthetic cyclic ecl2 peptide was used to mimic the distance constraint determined in a previous structural model of the NOP receptor. Data revealed the formation of a NOP-ecl2 complex. By specific ^{15}N labelling of selected amino acid residues of NOP, we confirmed the specific interaction between NOP and ecl2, notably by relaxation, coupling constant measurements, and chemical shift variations. Specificity of the interaction, as assessed from dynorphin competition experiments, appears to be brought about essentially by NOP's basic core. The interaction resulted in a conformational modification of ecl2, which plays a crucial role for the activation of GPCRs. These results highlight the role of the NOP receptor ecl2 in NOP binding selectivity. The observed structural modifications point out conformational changes occurring at an early stage of receptor activation.

P-451

Ultrafast dynamics of bond formation between heme and axial residues in six-coordinate heme proteins

M. H. Vos[1], A. Battistoni[2], L. Kiger[1], A. Desbois[3], E. Pilet[4], E. de Rosny[4], U. Liebl[1]
[1]Ecole Polytechnique/INSERM U799, France, [2]Univ. of Rome Tor Vergata, Italy, [3]CEA Saclay, France, [4]IBS Grenoble, France

Heme proteins are involved in many biological functions, including transfer and storage of O_2 and of heme, catalysis, electron transfer and signaling. One of the two potential heme iron axial bonds is almost invariably formed by a histine. The other, 'distal' site can remain unoccupied, bind (functionally or not) external small ligands, or bind a second amino acid residue. In recent years a rapidly expanding group of proteins has been studied in which a distal residue can be replaced by an external ligand; a process initiated by thermal dissociation of the Fe-residue bond. This bond can also be dissociated by a short light pulse[1].
We used femtosecond absorption spectroscopy to investigate the rebinding kinetics of the distal residue in a wide variety of six-coordinate heme proteins. These include mammalian cytochrome *c* (Met, soluble electron transfer protein, *c*-type heme), plant cytochrome b_{559} (His, membranous electron transfer protein, *b*-type heme), mammalian neuroglobin (His, globin, possibly involved in oxygen storage), E75 from *Drosophila* (Cys, nuclear receptor with heme domain) and the superoxide dismutase from *Haemophilus ducreyi* (His, heme attached between two subunits at the protein surface, possible heme carrier). Remarkably, all rebinding kinetics are dominated by a phase of $\sim$6 ps, indicating a barrierless process. The functional implications of this behavior, and signaling-related deviations, will be discussed.
1. S. Cianetti et al., JACS 126 (2004) 13932 - 13933.

O-452

Modeling and simulation of protein-protein recognition and binding

R. C. Wade
EML Research gGmbH, Heidelberg, Germany

Protein-protein interactions are central to biological function. We are developing and applying computational methods to address questions such as: Could these two proteins have the same binding partner? How do two proteins dock together? How quickly and tightly do they bind? How is binding affected if one of the proteins is mutated? I will describe the use of Brownian dynamics simulations to simulate the diffusional association of proteins to compute association rates, characterize diffusional encounter complexes and investigate the factors influencing the protein-protein binding process [1,2]. I will then present a computational procedure to predict the structures of protein-protein complexes by a combination of Brownian dynamics and molecular dynamics simulations constrained by biochemical data. Applications to structurally and functionally diverse protein complexes with different determinants of protein-protein binding, including enzyme-inhibitor, signal transduction and electron transfer [3] complexes, will be presented.
[1] Gabdoulline,R.R., Wade,R.C. Biomolecular Diffusional Association *Curr. Opin. Struct. Biol.* (2002) 12, 204-213.
[2] Spaar, A., Dammer, C., Gabdoulline, R.R., Wade, R.C., Helms,V. Diffusional encounter of barnase and barstar. *Biophys. J.* (2006) 90, 1913-1924.
[3] Karyakin, A., Motiejunas, D., Wade, R.C., Jung,C. FTIR studies of the redox partner interaction in cytochrome P450. The Pdx-P450cam couple. *BBA General Subjects* (2007) 3, 420-431.

Abstracts

– Protein-ligand interactions –

P-453

Insights into acetylcholine receptor gating from a high-resolution structure of bound acetylcholine

P. T. F. Williamson[1], B. H. Meier[2], A. Watts[3], K. Miller[4]
[1]School of Biological Sciences, University of Southampton, U.K., [2]Department of Physical Chemistry, ETH Zurich, Switzerland, [3]Biomembrane Structure Unit, Biochemistry Department, University of Oxford, U.K., [4]Harvard Medical School, Massachusetts General Hospital, Boston, U.S.A.

Understanding how small molecules interact and activate integral membrane proteins remains one of the key questions in membrane biology. Here we report how solid-state NMR measurements have been combined with docking studies to gain insights into how acetylcholine binds to the nicotinic acetylcholine receptor and subsequently activates it.
Solid-state magic-angle spinning NMR methods were employed to determine multiple high-resolution structural constraints for the agonist, acetylcholine, bound to its binding site on the nicotinic acetylcholine receptor. This enabled us to characterise at high-resolution the conformation of the ligand with the receptor binding site. Docking of the rigid ligand to a flexible receptor host based on the crystal structure of a homologous acetylcholine binding protein from *Lymnaea stagnalis* allowed us to produce a model for the ligand within the receptor binding site. Not only has this permitted the identification of key residues involved in high affinity ligand binding but the changes observed in binding site conformation upon ligand binding provide insights into mechanisms for receptor activation.

P-454

Impact of antigenic peptide dynamics on the mechanism of T-cell cross reactivity

K. Winkler[1], D. Narzi[2], A. Ziegler[3], R. A. Böckmann[2], U. Alexiev[1]
[1]Physics Department, Free University Berlin, [2]Theor.&Comp. Membrane Biology, Univ. des Saarlandes, Germany, [3]Inst. f. Immungenetik, Universitätsmedizin Charite, Germany

A single difference between HLA-B*2705 and HLA-B*2709 (Asp116His) is responsible for the emergence of distinct T cell repertoires in individuals harbouring either of these two alleles and is expected to be coupled to their differential association to ankylosing spondylitis (AS). Asp116 was found to form a salt bridge to p5Arg in the peptides pVIPR (RRKWRRWHL) and pLMP2 (RRRWRRLTV), promoting a non-canonical peptide binding mode. By using time-resolved fluorescence depolarisation and molecular dynamics simulation we investigated to what extent structural and dynamical features of the non-canonical forms of pVIPR and its viral homologous pLMP2 contribute to the postulated molecular mimicry between these peptides as a relevant phenomenon in AS pathogenesis. Our data reveal distinct dynamical differences: In contrast to pLMP2, the conformational flexibility of pVIPR sensed in the middle of the peptide (p6) is higher in B*2705 than in B*2709. In addition, pVIPR shows an enhanced flexibility in the C-terminal region when bound to B*2705, in contrast to the expectations from x-ray structure. These data, together with a slow diffusional mobility of the pVIPR C-terminal part, point to the existence of dual conformations of pVIPR when bound to B*2705, which probably undergo interconversion. As the structural/dynamical features of pLMP2 and pVIPR when bound to B*2705 are different, our results provide new insight into the mechanism of T-cell cross reactivity observed for these peptides.

P-455

Two-step binding mechanism of the CBC protein-RNA 5'cap interaction

R. Worch[1], A. Niedzwiecka[2], M. Jankowska-Anyszka[3], C. Mazza[4], J. Stepinski[1], E. Darzynkiewicz[1], S. Cusack[4], R. Stolarski[1]
[1]Division of Biophysics, Warsaw University, Poland, [2]Inst. of Physics, Polish Acad. of Sciences, Warsaw, [3]Faculty of Chemistry, Warsaw University, [4]EMBL, Grenoble, France

Several techiniques were applied to analyze the binding mechanism of human nuclear Cap Binding Complex (CBC) to mRNA/snRNA 5' cap, m^7GpppN, where m^7G denotes 7-methylguanosine and N is any nucleoside. The heterodimer CBC protein takes part in numerous gene expression processes.
As shown by *ab initio* quantum calculations, methylation at N(7) results in enhancement of the stacking interaction with the tyrosines Y20 and Y43. Novel cap analogues were synthesized for SPR experiments with wild type CBC and its selected mutants. The sensograms can be interpreted in terms of a two-step binding model. Replacement of Y20 and Y43 by alanine greatly reduces the favorable free binding energy of the first step, whereas mutation of Y138 significantly decreases the energy of the conformational rearrangement. Significance of the interaction between the second cap base and Y138 was also confirmed by fluorescence titration experiments. The results extend a two-step binding model that was proposed previously on the basis of X-ray diffraction studies. Fast association with Y43 and partial folding of the Y20-containing loop is followed by slower rearrangement of partially disordered C-terminal part of CBP20, that allows final docking of the cap.
Supported by Polish Ministry of Science and Higher Education N301 007 31/0154 and 2 P04A 033 28.

Abstracts

– Single molecule imaging and spectroscopy –

P-456

Actin-CCT; A single molecule study of chaperone mediated protein folding

G. M. Altschuler[1], D. R. Klug[1], K. R. Willison[2]
[1]Department of Chemistry, Imperial College London, U.K., [2]Cell and Molceular Biology, Institute of Cancer Research, U.K.

Understanding the extensive network of complex *in vivo* protein-protein interactions executing the cell cycle and controlling cell structure and function is a crucial aspect of cancer research. The eukaryotic chaperone CCT (Chaperonin Containing TCP-1) is positioned in the heart of this network as it folds the primary cytoskeletal components actin and tubulin and regulates the activity of major protagonists of the cell cycle pathway; CDH1, CDC20 and cyclin E. Structural and biochemical studies have characterised chaperone-substrate binding though little has been uncovered of the underlying system kinetics/thermodynamics. This is of great interest as the analysis of actin folding/unfolding suggests a 'thermodynamic chaperone' requirement; unassisted actin folding may be energetically unfavourable. It is hypothesised that CCT plays an 'active' role in protein folding, coupling specific substrate-binding interactions to ATP binding/hydrolysis-driven conformational changes. To test this model we have developed a system to examine CCT-actin kinetics at the single molecule level, to work towards extracting the thermodynamic parameters of chaperone-bound folding reactions. Individual CCT complexes have been immobilised via DNA tethering, allowing orientation of molecules $\sim$10nm above modified glass substrates. This method accomplishes the dual fundamental requirements of maintaining protein functionality and allowing sampling on CCT folding timescales (>100s). Single molecule substrate interactions (CCT-actin) have then been probed by TIRF microscopy, allowing analysis of binding and folding kinetics.

P-457

von Willebrand Faktor (vWF) membrane interaction

J. I. Angerer, D. M. Steppich, A. Wixforth, M. F. Schneider
University of Augsburg, Germany

Von Willebrand Factor (vWF) is the crucial protein for the initiation of blood clotting under high shear flow conditions. It has been shown that it exhibits a complex adhesion process with a conformational dependent binding to the substrate (endothelial cell wall). However, until today the binding site or mechanism remains unclear. Using fluorescence microscopy, AFM force spectroscopy and Differential Scanning Calorimetry (DSC) we examined the interaction and arrangement of vWF to different phospholipid membranes (neutral, charged, gel, fluid) under flow conditions. We therefore developed a new setup with a surface acoustic wave (SAW) pumping system for the fluorescence microscopy and DSC experiments which enabled us to perform adhesion experiments on both supported membranes and vesicles with coiled as well as the physiological active unrolled vWF molecules. For the AFM experiments vWF was bound tightly to a chemically treated AFM tip, which enables us to measure interaction forces in the pico-Newton regime. Furthermore we found a conformational dependent adhesion to protein free phospholipid membranes in both AFM force spectroscopy and DSC experiments. Based on our results we propose that vWF interacts strongly with the hydrophobic core of a membrane in the fluid phase and is not influenced by the electrostatic part of the membrane.

P-458

Imaging nucleosomes by scanning probe methods

F. Argoul, F. Montel, T. Roland, L. Berguiga, C. Moskalenko, P. Bouvet, H. Menoni, F. Mongelard, C. Vaillant, B. Audit, A. Arneodo
Laboratoire Joliot Curie, Ecole Normale Supérieure, 46 Allée d´Italie, 69364, Lyon, France

DNA is the physical support of genetic inheritance. Compacting DNA inside a very small volume is a general constraint for all living cells and its achievement remains an enigma both from structural and dynamical perspectives. Since the intrinsic structural and mechanical properties (bending and torsion) of the DNA polymer strongly depend on the sequence, the DNA sequence is likely to influence DNA interaction with structural proteins. This work has been performed in a transdisciplinary platform involving biochemists and molecular biologists, experimental physicists and theoretical physicists. We aim at making the link between DNA sequence and structural and mechanical properties of chromatine fiber and providing a better understanding of functional organization of genome. We report here two complementary experimental approaches which have been applied to study sequence effects on the DNA-histone interaction and on nucleosome mobility: scanning surface plasmon microscopy and atomic force microscopy.

P-459

Multiple CCR5 interacts constitutively with one CD4 at the cell surface: a vrFRAP and SPT approaches

A. Baker[1], A. Sauliere[1], G. Gaibelet[1], B. Lagane[2], S. Mazeres[1], F. Bachelerie[2], L. Salome[1], A. Lopez[1], F. Dumas[1]
[1]IPBS/ CNRS Toulouse, France, [2]Institut Pasteur,Paris, France

Fusion induced by human immunodeficiency virus type 1 (HIV-1) is a multistep process which requires that virus binds two distinct surface receptors of host cells: CD4 and CCR5. The binding of the gp120 viral protein to its receptors triggers structural changes exposing the viral gp41 protein which inserts into the membrane host cell and initiate the fusion of viral and cellular membranes.
Using a stationary approach (FRET), we have recently shown that CD4 and CCR5 receptors constitutively interact and that gp120 reinforces these associations. However, these molecules are in dynamic interaction and the knowledge of their mobility and confinement is required to a better view of the infection process.
Thus to go further, in this study, we have carried out two dynamic approaches. Using vrFRAP in living cells stably expressing CD4 and/or CCR5, we found that (1) a small fraction of these proteins is confined in micrometer domains, (2) constitutive CD4-CCR5 associations occur mostly outside of the confined domain, (3) these interactions involve several CCR5 per CD4.
Currently, a SPT approach is used, in order to have more accuracy on CD4 and CCR5 interaction. Kind of receptor (confined, directed...) involved in the interaction will be determined. Previously works seem to show that preferentially directed CCR5 receptors are in constitutive interaction with CD4.
These results allow us to propose that CD4-CCR5 pre-association is a prerequisite for HIV-1 infection to occur.

Abstracts

– Single molecule imaging and spectroscopy –

P-460

Quercetin effects on survival and delayed luminescence of hydrogen peroxide-treated yeast cells

I. Baran[1], C. Ganea[1], F. Musumeci[2], S. Tudisco[2], A. Scordino[2], S. Privitera[2], L. Lanzano[2], V. Baran[3]
[1]Biophysical Dept., UMF "Carol Davila" Bucharest Romania, [2]INFN - Lab. Naz. del Sud, Catania, Italy, [3]Dept. Theor. Physics and Math, Fac. Physics, UB, Bucharest, Romania

Quercetin is a natural antioxidant that in *Saccharomyces cerevisiae* yeast cells is able to permeate the cell wall and membrane. It is known that quercetin consistently increase cell survival after induction of DNA double-strand breaks by free radicals such as hydrogen peroxide. Due to its quenching effect on free radicals, we expect that quercetin has consistent effects on delayed luminescence (DL) of cells. This issue has not been investigated so far. Quantification of these effects can help answer the question on the origin of DL, which at the moment is still a matter of debate. Suspension yeast cell cultures are challenged to various doses of hydrogen peroxide in the presence or absence of quercetin. Cell survival is measured by assessing the colony-forming ability after a specific treatment. For DL determinations, biological samples are excited with a pulse of laser light and photons emitted by the system are detected with a photomultiplicator tube, set to count single photons. Spectral analysis of the emitted light is done with the use of seven filters that correspond to seven wavelengths which span the visible region (from 395 to 763 nm). Both the maximal intensity of emitted light and the kinetics of photon emission are measured. Survival and DL characteristics of treated cells are directly compared to those observed in control cultures. A consistent decrease in DL intensity is observed in quercetin-treated cells, suggesting that free radicals substantially contribute to DL emission.

P-461

Insights into the spectral versatility of fluorescent proteins by single molecule spectroscopy

C. Blum[1], A. Meixner[2], V. Subramaniam[1]
[1]Biophysical Engineering Group, University of Twente, The Netherlands, [2]Institut für Physikalische Chemie, University of Tübingen, Germany

Understanding the photophysics of fluorescent proteins is essential for accurate interpretation of the biological and biochemical processes illuminated by the fluorescent proteins as well as for the development of biosensors based on fluorescent proteins.We used spectrally resolved single molecule spectroscopy to analyse aspects of fluorescent protein photophysics that are not accessible by conventional ensemble spectroscopy. We were able to identify and characterize different subensembles and spectral forms of a range of fluorescent proteins. We could follow transitions between the different spectral forms on the single molecule level and draw conclusions on the underlying molecular origins. Fluorescent proteins are excellent systems to analyze the interaction between a chromophore and its nanoenvironment since the local environment of the emitting chromophore is precisely defined by the protein that encapsulates the chromophore. We find that for the fluorescent proteins studied, the width of the distribution of the single molecule emission maximum positions is strictly correlated with the flexibility of the chromophore nanoenvironment. Further we analyzed the fluorescence resonance energy transfer coupling of different chromophores within one protein tetramer. We find that in a fraction of the tetramers the different chromophores are not effectively coupled. We propose an interruption of the energy transfer chain within the multichromophoric system by proteins lacking a chromophore.

O-462

Changing the length of the dynein stalk alters step size but not directionality.

A. P. Carter, A. Yildiz, C. Cho, R. D. Vale
University of California San Francisco, U.S.A.

The cytoplasmic dynein head consists of a ring of AAA+ domains and a linker element that is essential for motility. The head attaches to the microtubule via a domain (MTBD) at the end of a 10-15 nm long stalk. This stalk is made up of approximately 14 heptad repeats of antiparallel coiled-coil and its length is universally conserved. We have determined the effect of changing the length of this coiled-coil on dynein motility using an artificially dimerised dynein expressed in *Saccharomyces cerevisiae*.

Dyneins with a shorter stalk could still move, albeit with slower than normal velocity. High-precision single molecule tracking of dynein movement showed that slow movement is at least partially due to a shorter step size of the motor. The results imply that the angle the stalk makes with the microtubule must change during motility. Robust movement of an elongated stalk was also observed when a sequence from another dynein stalk was inserted, but not if the sequence was from a non-stalk coiled coil (colicin-1a). This is consistent with the sequence of the dynein stalk being important for communication.

Changing the length of the stalk by 7 heptads would be expected to rotate the dynein head by 180 degrees with respect to the MTBD and hence change dynein directionality. However, surprisingly, all these constructs still moved towards the minus end of the microtubule. This suggests the orientation of the head is not required for dynein directionality and leads us to speculate that the direction of movement results from the angle the stalk makes with the microtubule during dynein's powerstroke.

P-463

EPR and spectrophotometric investigation of the antioxidant capacity of some romanian grapes and wines

C. Cimpoiu[1], A. Hosu[1], G. Damian[2], V. Miclaus[1]
[1]Babes-Bolyai University, Department of Chemistry, [2]Department of Physics, Cluj-Napoca, Romania

The high level of reactive free radicals occurring in oxidative stress conditions in living organisms have received considerable attention [1]. Plant phenolics play an important role in disease prevention, being able to neutralize free radicals [2]. Especially red grapes are known as antioxidant sources and their extracts are used as active ingredients in cosmetic and pharmaceutical industry [3]. The aim of our study was to investigate the antioxidant capacity of some ethanol extracts prepared from seeds and skins of different types of red grapes and corresponding commercial wines from Romanian Recas winery. The antioxidant activity was evaluated by UV-VIS and EPR spectroscopy using TEMPOL and DPPH free radicals. The changes in absorbance and in signals intensity were monitored. The experimental EPR spectra were double integrated and the relative concentrations of free radicals were determined.All samples reveal different antioxidant capacity depending on types of red grapes. The wines are less antioxidant than skins extracts. Seeds extracts show the highest antioxidant activity.

Bibliography: [1] Victor V. M., McCreath K. J. and Rocha M., Recent Patents on Anti-Infective Drug Discovery, 1, 17-31, 2006; [2] Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jimenes, L., Polyphenols: food sources and bioavailability. Am. J. of Clinical Nutrition, 79, 727-747, 2004; [3] Lopez-Velez, M., Martinez-Martinez, C. Del Valle-Ribes, F., The study of phenolic compounds as natural antioxidants in wine. Critical Review in Food Science and Nutrition, 43, 233-244, 2003

Abstracts

– Single molecule imaging and spectroscopy –

P-464

Rapid diagnosis by biomarkers measurements

M. Culea[1], M. Chiriac[2]
[1]Babes-Bolyai University of Cluj-Napoca, 1 Kogalniceanu str, 400084 Cluj-Napoca, Romania, [2]National Institute for Research and Development of Isotopic and Molecular Technologies, 65-103Donath str,Cluj-Napoca, Romania

An isotopic dilution GC-MS rapid method was developed for diagnosing inborn error of metabolism of some neonatal diseases. Small volumes of dry plasma or blood spots were used for neonatal blood screening for diagnosis of phenylketonuria and MSUD metabolic diseases. The blood samples were derivatized as trifluoroacetylbutyl esters and analyzed by gas chromatography coupled with mass spectrometry in the selected ionization monitoring (SIM) mode. Regression curves for standard amino acids are used for quantitative determination of valine, leucine, proline, phenylalanine and tyrosine, by using ^{15}N-isoleucine as internal standard. GC/MS analyses were performed on a Trace DSQ ThermoFinnigan quadrupole mass spectrometer coupled with a Trace GC gas chromatograph. Samples were separated on a Rtx-5MS capillary column, 30 m x 0.25 mm, 0.25μm film thickness, using a temperature program from 50^{o}C(1min), then 20^{o}C/min to 260 ^{o}C, 100^{o}C/min to 300 ^{o}C, in the selected ion monitoring (SIM) mode. In the SIM mode the following important ions from the mass spectra of Phe, Pro, Val, Leu and Tyr were used: m/z 91, 148, 204 for Phe, m/z 166 for Pro, m/z 168 for Val, m/z 182 Leu, m/z 203, 260, 316 for Tyr and m/z 183 for the internal standard. The following conditions were followed: transfer line temperature: 250^{o}C, injector temperature:200 ^{o}C; ion source temperature 250 ^{o}C; Splitter: 10:1. Electron energy was 70eV and emission current 100μA.

P-465

GFP-mut2 proteins in trehalose-water matrixes: spatially heterogeneous protein-water-sugar structur

L. D'Alfonso[1], M. Collini[1], G. Chirico[1], B. Campanini[2], G. Cottone[3], L. Cordone[3]
[1]Dipartimento di Fisica, Università di Milano Bicocca, Milano, Italy, [2]Dipartimento di Biochimica e Biologia Molecolare, Università di Parma, Parma, Italy, [3]Dipartimento di Scienze Fisiche e Astronomiche, Università di Palermo, Palermo, Italy

We report investigations on the properties of nano-environments around single GFP-mut2 proteins in trehalose-water matrixes. Single GFPmut2 molecules embedded in thin trehalose-water films were characterized in terms of their fluorescence brightness, bleaching dynamics, excited state lifetime and fluorescence polarization. For each property, sets of about 100-150 single molecules have been investigated as a function of trehalose content and hydration. Three distinct and interconverting families of proteins have been found, which are widely differing in terms of bleaching dynamics, brightness and fluorescence polarization, whose relative populations sizably depend on sample hydration.
The reported results evidence, at single molecule level, the simultaneous presence of different protein-trehalose-water structures whose rigidity increases by lowering the sample hydration. Such spatial inhomogeneity is in line with the well-known heterogeneous dynamics in supercooled fluids and in non-solid carbohydrate glasses, and gives a pictorial representation of the sharp, sudden, collapse/reorganisation of the above structures following uptake/release of water molecules.

P-466

Tailored ICAM1 patterned surfaces to study the nanoscale organization of the adhesion receptor LFA1

R. Díez-Ahedo[1], G.-J. Bakker[1], B. Joosten[2], C. G. Figdor[2], M. F. García-Parajó[1]
[1]Bionanophotonics, IBEC, Barcelona, Spain, [2]Tumor Immunology, Nijmegen Centre Molecular Life Sciences, The Netherlands

Lymphocyte function-associated antigen-1 (LFA1;αLβ2) is a leukocyte specific integrin transmembrane protein that mediates migration across the endothelium and within tissues. It also takes part of the immunological synapse by binding with high affinity with its ligand ICAM-1.
We have recently shown that integrin mediated adhesion depends not only on receptor occupancy but also on its nano-cluster organization on the cell membrane [1].
In order to get deeper insight on the mechanisms that control and regulate LFA-1 clustering we are applying on the one hand soft-lithography techniques to create ICAM-1 tailored surfaces that are able to mimic cell-cell interaction at the nanometer-scale, and on the other hand single fluorescence molecule techniques for optical inspection on living cells.
We have fabricated reproducible patterns with homogenous ICAM-1 density in the range of 10 to 1 μm on glass substrates, and we have used Total Internal Reflection (TIRF) microscopy to resolve clusters of LFA-1 transfected with Green Fluorescent Protein (GFP) in living L cells. Currently we are investigating the effect of ICAM-1 patterning on the LFA-1 cluster properties and also quantifying the number and density of LFA-1 clusters. A better control and study of receptor nanoclustering is a challenge for developing state-of-the-art biocompatible platforms.
[1] A. Cambi et al. Molecular Biology of the Cell, 17, 4270, 2006.

P-467

Static and dynamic flexibility of polythymine oligonucleotides

S. Doose, H. Barsch, M. Sauer
Applied Laser Physics & Laser Spectroscopy, University of Bielefeld, 33615 Bielefeld, Germany

Biopolymers, such as single-stranded DNA (ssDNA), are often described as semiflexible polymers or worm-like chains. We investigated static and dynamic flexibility of homogeneous ssDNA (polythymine), a model polyelectrolyte, in various conditions of ionic strength. Using fluorescence correlation spectroscopy we found that the hydrodynamic radius *Rh* scales with contour length according to a power law with an exponent between 0.5 and 0.7 depending on ionic strength *I*. With *Rh* being proportional to the square root of the persistence length *Lp*, we found that $Lp \sim I\hat{}m$ with m = (-0.22±0.01) for polythymine with 100 residues. We further studied end-to-end contact kinetics for polythymine with up to 10 residues by observing contact-induced fluorescence quenching between 3'-terminal guanine and 5'-terminal fluorophore. For comparison we performed Molecular Dynamics (MD) simulations with a force field that accounts for short-range interactions in vacuum, and determined the characteristic polymer properties end-to-end distance *R*, radius of gyration *S*, and persistence length *Lp* of various labeled and non-labeled polythymine derivatives. We found excellent agreement for the length dependences of simulated *S* and experimental *Rh* measured at 100 mM NaCl, revealing that electrostratic interactions are completely shielded in aqueous solution with such ionic strength. These results provide a benchmark for theories and MD simulations describing the influence of electrostatic interactions on polyelectrolyte properties, and thus help to develop a complete and accurate description of ssDNA.

Abstracts

– Single molecule imaging and spectroscopy –

P-468

Chemical modification of graphite surface for ultra low current STM of DNA

E. Dubrovin[1], J. W. Gerritsen[2], A. Lomonosov[1], S. Speller[2], I. Yaminsky[1]

[1]Department of Physics of Polymers and Crystals, Faculty of Physics, Moscow State University, Leninskie Gory, 1/2, Moscow, 119992, Russia, [2]Institute for Molecules and Materials, University of Nijmegen, Toernooiveld, 1, Nijmegen, 6525 ED, Netherlands

Since the end of 1980-s a lot of attempts to acquire atomic resolution images of DNA using scanning tunnelling microscopy (STM) have already been made (1) but they all were recognized either as artefacts or non-reproducible ones (2). Last years it was already shown the possibility of acquiring submolecular resolution on different soft polymer molecules and on the short DNA fragments using STM (3-4). One of the remaining problems is to find appropriate methods to align long DNA molecules in order to get physical access to the primary structure of the molecule by the nanoprobe. In this work we found the procedure of modification of graphite surface with different alkylamines. This modification leaves the surface flat enough, allows adsorb DNA and make STM study of single DNA molecules on it.

Acknowledgements. This work is supported by INTAS project N 014-6323.

(1) Nature, 1990, v.346, 294;
(2) Science, 1991, v.251, 640;
(3) J. Vac. Sci. Technol. B, 1999, v.17, 1313;
(4) Surf. Sci. Lett., v.539, 2003, L531.

P-469

Setting up new tools for biophysical studies of Hemagglutinin-mediated membrane fusion

F. Eghiaian, P. Le Tissier, J. E. Corrie, J. J. Skehel, C. Veigel

National Institute for Medical Research, NW7 1AA, London, U.K.

The influenza Hemagglutinin (or flu HA) is responsible for immobilising influenza virus particles on the surface of the host cell and for fusion between the host cell and the viral membranes. Following endocytosis, HA-mediated membrane fusion occurs during intracellular transport, when viral surface proteins are being exposed to low pH in the endosomes. Low pH triggers a conformational change upon which the functional segment of HA named "fusion peptide" becomes solvent exposed and contacts the host's membrane. The mechanisms by which the flu HA facilitates subsequent merging of lipid membranes and opening of a fusion pore in the merged membrane are unclear: in particular it is not known how many HA molecules are needed for membrane fusion or whether structural intermediates of HA contribute to different stages of fusion. In order to obtain a more detailed understanding of the basic mechanisms underlying HA-mediated membrane fusion we use a combination of molecular biology and single molecule biophysical techniques. We are expressing mutant HA for single molecule imaging of the fusion process. We also use a photoactivatable caged proton to allow for a fast pH jump, in order to study the fusogenic conformational change of single HA molecules at high time resolution. *(Supported by MRC and The Royal Society)*

P-470

Proteomics Analysis of Medicago sativa under TNT stress

S. Fallahi[1], G. Hosseini Salekdeh[3], M. Habbibi-Rezaie[2], M. Khayami[1], R. Haydari[1]

[1]Department of Biology, University of urmia, [2]School of Biology, University College of Sciences, University of Tehran, [3]Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

Soil and groundwater at sites throughout the world were contaminated in the past century by manufacturing, processing, and storage of explosives. TNT is also present in the environment as a result of decommissioning activities and through field usage and disposal activities such as open burning.

Certain plant species show the ability of removing of TNT from soil which is followed by its accumulation in the plant tissue. This offers a potential mean for removing these compounds from the environment. Phytoremediation is an innovative technology that using trees, grasses and other plants to clean out the environment and to remediate hazardous waste sites.

Proteomics is a powerful tool for detection and analysis of stress responsive proteins and genes. *Medicago sativa* seeds are exposed to TNT in silica, and then was investigated the stress effects on protein expression pattern. After 30 days, shoot proteins were extracted by TCA method and analyzed through 2D-electrophoresis followed by ESI-Q-TOF MS/MS analysis. Totally, more than 710 protein spots were detected in 2D gels respectively by software package. 107 proteins showed significant response to stress among which, 6 and 3 proteins were switched on and off respectively.

P-471

Direct visualisation and kinetic analysis of actin polymerisation using TIRF microscopy

J. Feng[1], D. S. Ushakov[2], M. A. Ferenczi[2], S. B. Marston[1]

[1]NHLI, Myocyte Systems Biology, Imperial College London, U.K., [2]BMS, Imperial College London, U.K.

Actin filaments were formed by elongation of pre-formed nuclei (short crosslinked actin-HMM complexes). 2μg/ml nuclei were infused into a standard *in vitro* motility cell, followed by 2 washes in 1mg/ml BSA. Polymerisation was initiated by mixing monomeric actin (0.05-0.75 μM) with 150nM TRITC-phalloidin, 50mM KCl, 4mM $MgCl_2$ and 1% methylcellulose. The mixture was immediately infused into the motility cell and fluorescence was observed in the TIRF microscope (525nm excitation). By using TIRF illumination we could see actin filaments at high contrast despite the presence of 150nM TRITC in the solution. Actin filaments showed rapid bending and translational movements due to Brownian motion but the presence of the methylcellulose polymer network constrained lateral movement away from the surface. Both the length and the number of filaments increased with time. Some filaments did not change length at all and some filaments joined up end-to end (annealing). We did not see any decrease in filament length or filament breakage. The rate of polymerisation increased with increasing actin monomer concentration. For quantitative analysis of polymerisation time course we measured the contour length of all the filaments in a frame at a series of time points and also tracked the length of individual filaments over time. Elongation rate was the same measured by both methods (0.56μm/min at 0.1μM actin) and was up to 10 times faster than previously published measurements. The annealed filament population reached 30% of the total after 40 minutes. Total polymerisation rate (length x number) increased linearly with actin concentration and critical concentration was less than 20nM.

Abstracts

– Single molecule imaging and spectroscopy –

P-472

Quantitative force calibration in viscoelastic media and living cells

M. Fischer[1], A. Richardsson[2], N. Reihani[2], L. Oddershede[2], K. Berg-Sørensen[1]

[1]Department of Physics, Technical University of Denmark, [2]Niels Bohr Institute, University of Copenhagen, Denmark

Over the last years, optical manipulation of single bio-molecules have demonstrated its strength, with precise measurements reaching resolutions of Angstroms and femtoNewton in *in vitro* experiments. Optical tweezers as a tracking tool have also demonstrated their use in *in vivo* systems. Quantititative force measurements in vivo require, however, the development of methods for characterization of the optical trap in the cytoplasm. We discuss the prospects for mechanical measurements of forces in *in vivo* systems and present a suggestion for a protocol of experiments and data analysis to characterize an optical trap in viscoelastic media and the cytoplasm of a living cell. First experimental results with the method will be shown too.

P-473

Chromatin at the nanolevel: FRET spectroscopy and multiparameter detection of individual nucleosomes

A. Gansen[1], F. Hauger[1], K. Toth[1], S. Felakyan[2], C. A. Seidel[2], J. Langowski[1]

[1]German Cancer Research Center, Im Neuenheimer Feld 580, 69120 Heidelberg, Germany, [2]University of Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany

The local structure and dynamics of nucleosomes affects the compaction into the higher order chromatin structure, which is an important mechanism of gene regulation. We utilize a custom build confocal SMD system to investigate the dynamics within individual nucleosomes diffusing free in solution. Nucleosomes with energy transfer between 20 and 80 % were prepared from DNA labelled with Alexa488 and various acceptor dyes (Rhodamine X, Alexa594). We describe our measures to ensure sample integrity under SMD conditions..

The different positioning behaviour of two DNA sequences (Widom-601 and -612) was detected as separate subpopulations for internal labelled nucleosomes. This is currently further investigated using multiparameter detection. So far we were able to observe a selective dissociation at increased salt levels causing a redistribution between two nearby FRET states. Besides, catalytic repositioning, known to affect the nucleosome conformation, can be monitored using end-labelled nucleosomes. The presence of free DNA, showing a non vanishing FRET efficiency, limits the resolution in the low FRET regime. By analysing the Proximity Ratio instead of the FRET efficiency we observed a significant repositioning of end-labelled nucleosomes upon thermally induced and enzymatic repositioning. This approach will be extended to investigate the dynamics of remodelling factors in more detail.

O-474

Single molecule NSOM microscopy resolves distinct nano-scale compartments on the cell membrane

M. F. Garcia-Parajo[1], A. Cambi[2], G.-J. Bakker[1], M. Koopman[3], B. Joosten[2], C. G. Figdor[2]

[1]IBEC-Institut de Bioenginyeria de Catalunya, Barcelona, Spain., [2]Tumor Immunology, Nijmegen Center for Molecular Life Sciences, NL, [3]Applied Optics, TNW&MESA+, U. Twente, NL.

Recruitment of proteins to lipid domains known as rafts has been proposed as important mechanism to regulate their activity on a variety of cells. However, the association of transmembrane receptors with lipid rafts remains largely debated, and a direct visualization of these interactions has been challenging. We have exploited single molecule high-resolution near-field scanning optical microscopy (NSOM) in *aqueous* conditions to resolve for the first time individual lipids and receptor domains with an accuracy of 3-7 nm. We show that the glycosphyngolipid GM1, a major lipid raft component, organizes in nano-domains of $\sim$10-150nm in diameter on monocytes and dendritic cells. We further demonstrate that the putative raft-associated transmembrane integrin LFA-1 pre-organizes in nano-clusters spatially distinctive but significantly proximal to those of GM-1, with 20-25% of LFA-1 being within < 50nm proximity to GM1. In contrast, we show that the non-raft associated transferrin receptor is excluded from GM1 nanodomains. These remarkable results suggest that proximal but distinct compartmentalization occurs at the nm scale having direct consequences on the current view of rafts as stable protein-recruiting platforms. Different current models to explain our data as well as recent experiments on the dynamics of individual LFA-1 clusters and their potential interaction with lipid rafts will be presented.

P-475

Towards SNARE-induced fusion of giant unilamellar vesicles

A. J. García-Sáez[1], H. Hartmann[1], K. Bacia[1], A. Stein[2], J. Reinhard[2], P. Schwille[1]

[1]Biotechnologisches Zentrum der TU Dresden, Dresden, Germany, [2]Max-Planck Inst. for Biophysical Chemistry, Göttingen, Germany

SNARE proteins are essential components of the membrane fusion machinery in eukaryotic cells. The mechanism of action is still controversial, but the current model considers that SNARE proteins constitute the minimal fusion machinery. *In vitro* experiments show membrane fusion of large unilamellar vesicles induced by complementary SNAREs reconstituted in opposing membranes. Giant unilamellar vesicles (GUVs) are excellent model systems for the study of membrane processes with optical microscopy. With sizes in the range of microns, they form stable free-standing bilayers whose lipid composition can be controlled. Such a system would allow direct imaging of SNARE-induced membrane processes, with the possibility of stepwise increasing biological complexity. Hence, they provide a valuable tool to gain a deeper insight into the mechanism of SNARE-induced membrane fusion.

We have successfully reconstituted fluorescently labeled SNARE proteins into GUVs and studied them with confocal microscopy and FCS/FCCS. Direct observation of the interaction among Syntaxin 1, Synaptobrevin 2 and SNAP-25 in opposing membranes of GUVs suggests specific adhesive action by the SNAREs. Our results show interaction of opposing membranes over large areas, with accumulation of the complementary SNAREs at contact surfaces and at high curvature regions. Interestingly, this process can be inhibited by blocking the Syntaxin 1-containing GUVs with an unlabeled soluble form of Synaptobrevin 2.

Abstracts

– Single molecule imaging and spectroscopy –

P-476

Mechanically Induced Helix-Coil Transition in Biopolymers and their Gel Networks

J. L. Gornall, S. Courty, E. M. Terentjev
Cavendish Laboratory, University of Cambridge, U.K.

In recent years, a new field has emerged at the interface of physics and biology, aiming to explore structure and responses at molecular length-scales. Many single molecule experiments have been performed to measure forces generated by biopolymers and filaments, and their response via spectacular force-extension relationships. Such experiments reveal information about structural transitions that may occur on extension. We have used a new macroscopic approach, combining mechanical deformation of a biopolymer network with simultaneous probing of the internal structure via optical rotation or X-ray diffraction. A gelatin gel was our model system of a helix forming biopolymer network. Gelatin forms transparent gels containing extended physical cross-links, characteristic of the native collagen triple helix. The cross-links are separated along the chain contour by residues still in the coil sate. By imposing an extension on a chain which is above, but close, to its spontaneous helix-coil transition one can stimulate the helical state by reducing the randomizing effect of chain entropy. The net optical activity of the gel is proportional to the fraction of helical domains. Assuming affine mechanical deformation, we find a non-monotonic relationship between the fraction of helical domains, determined by optical rotation, and the externally imposed end-to-end distance of the chains. X-ray diffraction measurements confirm the increase in the fraction of helical domains on extension. The results are in agreement with the theoretical model of secondary helices induced by chain end-to-end stretching in amorphous coil regions of the chain.

P-477

Effects of exposure to 50Hz electromagnetic field on oxidant stress and antioxidant enzyme activities in the brain tissue of guinea pigs

G. Güler, N. Seyhan
Gazi University Faculty of Medicine/Biophysics Department, Ankara, Turkey

The exposure due to electric and magnetic fields in the ELF range arises from a wide variety of sources. The most frequented frequencies are 50 and 60 Hz and their harmonics, often called power frequencies. Despite many studies, the evidence as to whether ELF fields are detrimental to health remains controversal, and the debate continues. Within these debates, a hypothesis supported by most of the scientists is that EMF extends the lifetime of free radicals, and increases their concentrations, hence oxidative stress, which leads to possible adverse effects on cell functions and in folllowing process enhances the probability of damages to the living systems. The aim of this study was the evaluation of the influences of 50 Hz electric field of different strengths of 2 kV/m, 2.5 kV/m, 3 kV/m, 3.5 kV/m, 4 kV/m, 4.5 kV/m and 5 kV/m in both horizontal and vertical directions on free radical production and on antioxidant enzymes activity in brain tissue of guinea pigs. For this purpose, we aimed to assess the possible malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), myeloperoxidase (MPO), adenosine deaminase (ADA) and xanthine oxidase (XO) in ELF-electric field exposed guinea pigs. The results of the study showed that 50 Hz extremely low-frequency-electric fields, applied both horizantally and vertically with different strengths, did not effect the oxidant stress and antioxidant enzyme acitivities in statistically significant level ($p>0.05$) in brain tissue of exposed guinea pigs in this experimental conditions.

P-478

Fluorescence microscopy with the help of computational spectacles

R. Heintzmann[1], L. Hirvonen[1], K. Wicker[1], O. Mandula[1], K. A. Lidke[2]
[1]King´s College London, Randall Division, London, U.K., [2]Univ. of New Mexico, Albuquerque, NM, U.S.A.

Computers are an invaluable tool for fluorescence microscopy. They assist in the whole process from data acquisition to parameter extraction.
Some microscopy methods such as structured illumination, axial tomography or Pointillism would be unthinkable without the help of a computer. The notion is that the acquired data needs to contain the maximum information about your object but does not any longer need to be directly interpretable by a human observer. For fluorescence microscopy this will be exemplified:
In linear and non-linear Structured Illumination (SI) the sample is illuminated with a number of different patterns of light [1-4]. See Hirvonen et al. for details (#285, this volume). Experimental datasets and reconstructed results from linear SI data, demonstrating a resolution improvement of up to a factor of two over standard widefield microscopy are presented. A further approach to high resolution imaging is the localization of multiple particles in an image. This approach was named Pointillism [5]. Experimental data with particle separation based on independent component analysis will be presented.

1) R. H. and C. Cremer. *Proceedings of SPIE* , **3568**:185-196, 1999.
2) R. H. and P.A. Benedetti, *Applied Optics* **45**, 5037-5045, 2006.
3) R. H., T.M. Jovin, and C. Cremer. *JOSA A*, **19**, 1599-1609, 2002.
4) R. H.. *Micron*. **34**, 283-291, 2003.
5) K.A. Lidke et al. *Opt. Exp.* **13**, 7052-7062, 2005.

P-479

Spatial membrane heterogeneity investigated by EM-CCD camera based TIR-FCS

B. Kannan[1], M. M. Kumar[1], R. Kraut[2], T. Wohland[1]
[1]National University of Singapore, 3, Science Drive 3, Chemistry Department, Singapore 117543, Singapore, [2]Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, #04-01 Nanos, Singapore 138669, Singapore

A spatially-resolved fluorescence correlation spectroscopy (FCS) measurement system with total internal reflection (TIR) excitation has been constructed using an electron-multiplying charge-coupled device (EMCCD) camera. This system enables multiplexing of FCS measurements in TIRF configuration combined with imaging which finds immediate applications in the study of artificial lipid bilayers and cell membranes. The best time resolution is 4 ms for an ROI consisting of 20 lines in the CCD, independent of the ROI width. It decreases to 4.8 ms for 40 lines and to 35 ms for the full 512 lines. Cross-talk between neighboring pixels can be measured up to a distance of 5 pixels (corresponding to 1.4 μm). Therefore the system can measure up to 400 independent points at maximum time resolution. In this work we characterize different diffusion in artificial supported bilayers as well as in biological cell membranes. As an example for its application we examine the distribution of concentration and mobility of different membrane markers in artificial bilayers and live cells. Rho-PE, a fluorescent lipid analog is measured in POPC bilayers and as a standard system. This distribution is compared with transmembrane proteins in CHO cells, and raft and non-raft markers in neuroblastoma cells. The distributions show large differences depending on whether diffusion is measured on artificial bilayers or biological membranes, and the proposed location of the probe on and within the membrane.

Abstracts

– Single molecule imaging and spectroscopy –

P-480

Single molecule studies on individual metal complexes

A. Kiel[1], J. Kovacs[2], A. Mokhir[2], R. Krämer[2], D.-P. Herten[1]
[1]Physikalisch-Chemisches Institut, Heidelberg University, Im Neuenheimer Feld 253, D-69120 Heidelberg, Germany, [2]Anorganisch-Chemisches Institut, Heidelberg University, Im Neuenheimer Feld 271, D-69120 Heidelberg, Germany

The dynamics of individual copper(II) complexes with the bidentate ligand 2,2'-bipyridene-4,4'-dicarboxyacid was followed by time-resolved single molecule fluorescence spectroscopy. Based on intramolecular quenching of the fluorescent dye tetramethlyrhodamine by copper(II) complexes, changes in fluorescence intensity can be associated to the binding state of copper(II) ions to the covalently attached ligand. Immobilization of the probe and subsequent imaging allowed localization and time-resolved observation of individual dye-ligand conjugates. The time-resolved single molecule studies revealed fluctuations in fluorescence emission oscillating between distinct high- and low-fluorescent states. At a concentration of 2 μM $CuSO_4$ the durations of the highly-fluorescent on-states are in the order of 1 s decreasing linearly with increasing copper(II) concentration. In contrast to that, the durations of the low-fluorescent states which are also in the order of 1 s remain unchanged with increasing copper(II) concentrations. The observed linear dependence of the on-time durations from the copper(II) concentration suggest that individual association and dissociation events of copper(II) ions to the ligand are observed. By statistical evaluation of the recorded on- and off-time durations the kinetics of the complex formation and dissociation can be determined in thermodynamic equilibrium.

P-481

Molecular recognition at membrane surfaces: Coupling of G-proteins to the receptor rhodopsin

T.-Y. Kim[1], M. Moeller[1], K. Winkler[1], H. Ujii[2], B. Muls[2], J. Hofkens[2], U. Alexiev[1]
[1]Freie Universitaet Berlin, Department of Physics, Berlin, Germany, [2]Katholieke Universiteit Leuven, Department of Chemistry, Leuven, Belgium

Ligand-activation of G-protein coupled receptors (GPCRs) proceeds by multiple conformational changes leading to G-protein mediated signaling in physiological diverse processes. Despite numerous investigation, the precise molecular mechanism underlying the recognition of activated receptors (R*) by their cognate G-proteins is still poorly understood. The design of an intramolecular fluorescence based folding sensor into a key binding element of the catalytic transducin subunit and application of 2-dimensional time-resolved fluorescence spectroscopy (1) allows us to monitor the binding and real-time folding of that binding element upon engagement with the light-activated membrane receptor. Using the heterotrimeric G-protein transducin and transducin peptides labeled with a fluorescence dye in combination with wide field microscopy we monitor the time-course of light-activated binding of single transducin molecules to native rhodopsin membranes. We will discuss the observed changes in distribution and mobility of the G-protein transducin at the membrane surface as obtained by single particle tracking, as well as the protein-protein interaction before and after activation of the receptor.

(1) Kim, T.-Y., Winkler, K. and Alexiev, U. (2007), Photochem. Photobiol., 83:378-384

P-482

Toll-like receptor 9 and its ligand CpG-DNA studied with AFM and FCS

D. C. G. Klein[1], E. Latz[3], T. Espevik[2], B. T. Stokke[1]
[1]Dept. of Physics, NTNU, Trondheim, Norway, [2]Dept. of Cancer Research and Molecular Medicine, NTNU, Trondheim, Norway, [3]Dept. of Medicine, Div. of Infectious Diseases and Immunology, Univ. of Massachusetts Med. School, Worcester, USA

Toll-like receptor 9 recognizes DNA that contains non-methylated CpG motifs. These motifs are prevalent in bacterial DNA, but not in vertebrate DNA. The recognition initiates innate and acquired immune responses. Synthetic oligodeoxynucleotides (ODN) that stimulate TLR9 are currently being used in human clinical trials for cancer therapy and as vaccine adjuvant. Immunostimulatory ODN with different secondary and tertiary structure have a different stimulatory effect. A-class ODN stimulates interferon production, while B-class ODN activates B-cells. A-class has poly-G ends that self-associate into quadruplex structures via Hoogsteen base pairing, and contains a palindromic sequence. B-class is linear and does not contain a palindrome.

We use AFM and FCS to investigate A and B class ODN and their interaction with TLR9. Our AFM images of A-class CpG-DNA confirm the nanoparticle structures that were seen by Costa et al., while B-class does not form these higher order structures. FCS auto-correlation measurements in solution of labeled A-class resulted in a longer average diffusion time than for B-class. We investigated the binding of A and B class CpG-DNA to fusion proteins of the ectodomain of human TLR9. Measured diffusion times increased considerably upon addition of TLR9 fusion protein to both A and B class DNA, which confirms the binding of A and B class CpG-DNA to the TLR9 fusion proteins.

P-483

2D vibrational spectroscopy of peptides and proteins: fingerprinting and strutctural analysis

D. R. Klug[1], L. M. C. Barter[1], P. M. Donaldson[1], F. Fournier[1], E. M. Gardner[1], I. R. Gould[1], R. Guo[1], K. R. Willison[2]
[1]Imperial College London, Dept. of Chemistry, UK, [2]Institute of Cancer Research, Center of Cell. & Molec. Bio., UK

We have been developing and applying a particular variant of coherent two dimensional infrared spectroscopy (2DIR) for the study of protein identity, structure and function. The method is broadly analogous to 2DNMR which measures spin-spin coupling. In our case cross-peaks appear in the spectrum when both IR beams are in resonance with two vibrational modes which are also coupled together.

We show specific vibrational signatures of tyrosine and phenylalanine for different peptides. The ratio of these features to an internal vibrational peak (CH_2) taken as a reference show to be directly correlated to the amino-acids content in the different peptides. This result demonstrates the possibility of quantifying the amount of amino-acids in peptides and proteins and its development as a tool for high throughput protein identification. A structural analysis of bacteriorhodopsin active site is also presented. The photocycle of this chromophore is well documented and it is known that different conformers exist during the different steps of the photocycle. Strong cross-peaks specific of the chromophore are detected without interfering signal from the surrounding protein, via a triple resonance method. We show that both the ground state and the isomerised form of the retinal can be identified and distinguished and that this interpretation is supported by quantum mechanical calculations of the bacteriorhodopsin spectrum demonstrating the utility of this version of 2DIR for the analysis of enzyme active sites.

Abstracts

– Single molecule imaging and spectroscopy –

O-484

Biomedical applications of scanning ion conductance microscopy

Y. E. Korchev
Imperial College Faculty of Medicine, London, U.K.

Research in nanotechnology began with applications outside of biomedicine and is based on discoveries in physics and chemistry. This is because it is essential to understand the physical and chemical properties of molecules or complexes of molecules in order to control them. The same holds true for the molecules and structures inside living tissues. We need more detail on the physical properties of intracellular structures, and how biology's molecular machines are built. This basis, in turn, will enable drug development and therapy.

This approach ultimately requires the development of novel biophysical methods. For example, image living and functioning cells on the nanoscale and make quantitative measurements down to the level of individual molecules and their complexes. We have recently pioneered the development of an array of new and powerful biophysical tools based on Scanning Ion Conductance Microscopy that allow quantitative measurements and non-invasive functional imaging of single protein molecules in living cells. Scanning ion conductance microscopy and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial resolution of living and functioning cells, but also in the rich combination of imaging with other functional and dynamical interrogation methods. These methods, crucially, will facilitate the study of integrated nano-behaviour in living cells in health and disease.

P-485

Single molecule mass spectrometry using a solitary nanopore

O. V. Krasilnikov[1], J. W. F. Robertson[2], C. G. Rodrigues[1], V. M. Stanford[3], K. A. Rubinson[4], J. J. Kasianowicz[2]
[1]Laboratory of Membrane Biophysics, Department of Biophysics and Radiobiology, Federal University of Pernambuco, Recife, PE, Brazil, [2]National Institute of Standards and Technology, Electronics and Electrical Engineering Laboratory, Gaithersburg, MD, USA, [3]National Institute of Standards and Technology, Information Technology Laboratory, Information Access Division, Gaithersburg, MD, USA, [4]Department of Biochemistry and Molecular Biology, Wright State University, Dayton, OH, USA

Protein ion channels are nanometer-scale pores that facilitate ion and biopolymer transport across cell membranes. By virtue of their ability to interact with a wide variety of physical and chemical stimuli, they also function as sensors for specific molecules. We demonstrate proof-of-concept for the use of a single channel to discriminate between differently-sized poly(ethylene glycol) molecules (PEGs) in a polydisperse sample. Specifically, we show that the partitioning of individual PEG molecules into the pore causes single channel current blockades with amplitudes and lifetimes that are characteristic of that particular molecule's mass. The resolution is better than a single polymer repeat unit and the conductance-based mass spectra correlate with MALDI-TOF mass spectra of the same samples. The results show that single protein nanopores have the potential for non-destructive measurement of the detailed physical properties of individual molecules in aqueous solution.

P-486

Direct observation of an active human telomerase monomer at the single molecule level

H. Li, D. Alves, R. Codrington, A. Orte, D. Klenerman, S. Balasubramanian
Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK

Human Telomerase possesses two essential components: A catalytic protein subunit, hTERT, which extends its substrate DNA, and an RNA component, hTR, which provides the template for this extension. We have studied the minimal component composition of recombinant human telomerase using a single-molecule fluorescence technique. *Two-colour coincidence detection* (TCCD), which employs confocal blue and red lasers for the detection of dual-labelled biomolecules, has been utilised to observe the interaction between the core components within single telomerase complexes in solution. We systematically fluorescently labelled pairs of these components prior to reconstitution in rabbit reticulocyte lysate. We then directly observed single particles possessing hTR, hTERT and telomeric substrate DNA. This assembled material, which we show to be active, comprises an absolute subunit stoichiometry of one hTR, one hTERT and one substrate binding site moiety per telomerase complex. The interaction between hTR and hTERT was stable over 24 h, which correlated well with stable telomerase catalytic activity during the same period. In contrast, telomeric DNA was seen to dissociate from telomerase over this same time period. To the best of our knowledge this is the first direct observation and elucidation of functional human telomerase at the single molecule level.

P-487

Towards single molecule imaging of hydrophilic proteins via AFM

R. Marangoni[1], V. Barison[1], M. Cossi[2], R. Gottardi[3], R. Raiteri[3]
[1]ProteoGen Bio S.r.l, Pisa, Italy., [2]Dipartimento di Scienze dell´Ambiente e della Vita, Università del Piemonte orientale "Amedeo Avogadro", Alessandria, Italy, [3]Dipartimento di Ingegneria Biofisica ed Elettronica, Università di Genova, Italy.

AFM has been used on hydrophobic proteins or macromolecular complexes and images have been obtained. Soluble proteins are, on the other hand, moved by the interaction with the probe and cannot be scanned. ProteoGen Bio S.rl. has patented a method able to block a protein on a surface without significantly alter its 3D structure. The present work is aimed at exploring which experimental observation conditions can be set to make this achievement useful to obtain protein images at a resolution interesting for biologists. We studied, both theoretically and experimentally, a well known hydrophilic protein: the Trypsin. The computer approach included the development of ad-hoc electron-density simulation algorithms. The binding technology by ProteoGen Bio has been proved to efficiently immobilize Trypsin on the silicon slab, making it observable with the AFM. The computational approach is able to a priori determine the size of the optimal AFM tip to be used for imaging an assigned protein, thus maximizing the quality of the experimental image. Moreover, it is possible to quantify the decrease in the experimental image quality dependently on a given tip size. The studies performed on Trypsin with a tip with 5 nm radius of curvature showed that, by using our approach, it is possible to identify the molecule orientation (trihedral angle). Our approach is naturally extensible to compute other characteristics of the observed molecule, such as the 3D structure preservation.

Abstracts

– Single molecule imaging and spectroscopy –

P-488

INS spectroscopy study of Pt(II) and Pd(II) polyamine complexes displaying anticancer activity

M. P. Marques[1], J. Tomkinson[2], L. A. Batista de Carvalho[1]

[1]Molecular Physical-Chemistry, University of Coimbra, Portugal, [2]ISIS Facility, The Rutherford Appleton Laboratory, Chilton, U.K.

Pt-based antitumour drugs have been the target of intense research since Rosenberg's discovery of cisplatin (*cis*-$(NH_3)_2PtCl_2$). Their antitumour properties are based on selective interactions with DNA. However, a simple change in structure can drastically affect this cytotoxic activity. Pt(II) and Pd(II) complexes with two or three metal centres and aliphatic polyamines as bridging linkers, constitute a new class of third-generation drugs of great potential clinical importance.

This work reports an inelastic neutron scattering (INS) spectroscopy study of Pt(II) and Pd(II) chelates with biogenic amines. Good quality INS spectra were obtained from *ca.* 250 mg of compound, which is the smallest sample of a hydrogenous compound for which a successful INS interpretation has been reported. These INS spectra were completely assigned, in the light of DFT calculations and optical vibrational spectroscopy (Raman and FTIR) data. The cytotoxic properties of these complexes were previously evaluated [1-3], in view of gathering information on the *structure-activity relationships (SAR's)* ruling their biologial activity.

1. M.P.M. Marques *et al.*, *BBA (MCR)* **1589** (2002) 63.
2. L.J. Teixeira *et al.*, *J.Med.Chem.* **47** (2004) 2917.
3. S.M.Fiuza *et al., Letters in Drug Design and Development* **3** (2006) 149.

Finantial support from FCT (Portugal) – POCTI/47256/QUI/2002 (co-financed by the EC fund FEDER)- is acknowledged.

P-489

Single fluorophore tracking of P-selectin-EGFP during exocytosis of Weibel-Palade bodies

G. I. Mashanov, L. Knipe, J. E. Molloy, T. Carter
MRC, National Institute for Medical Research, London, UK

Weibel Palade bodies (WPB) are endothelial cell-specific secretory organelles that contain the leukocyte adhesion molecule P-selectin. WPB exocytosis delivers P-selectin to the cell surface in response to vasculature injury or inflammation. Little is known about the dynamics of P-selectin in the endothelial cell plasma membrane. The mobility of P-selectin-EGFP in the plasma membrane of human umbilical vein endothelial cells (HUVEC) under resting conditions and during delivery into the membrane following WPB exocytosis was studied. P-selectin-eGFP was transiently expressed in HUVEC by nucleofection and imaged using total internal reflection fluorescence microscopy. Using single fluorophore tracking we measured the lateral mobility of P-selectin, constitutively expressed at the plasma membrane under resting conditions ($0.075\pm0.09\mu m^2/s$, n=1537). Distribution of D_{lat} of the individual molecules showed two types of mobility: freely moving molecules and molecules with severely restricted mobility. 48hr post-nucleofection, P-selectin was found predominantly within WPB with few detectable single fluorophores on the plasma membrane. Following WPB exocytosis, evoked by ionomycin ($1\mu M$), the number of detectable single fluorophores on the membrane increased significantly. The majority of single fluorophores detected during WPB exocytosis were tracked in the first few seconds following exocytosis, within a $2\mu m$ radius of the point of fusion. The lateral mobility of secreted P-selectin at the site of WPB fusion was $\sim0.12\mu m^2/s$ (n= 264 objects). Acutely secreted P-selectin, at the WPB release site, is more mobile than constitutively expressed P-selectin.

O-490

Probing fast conformational dynamics of biomolecules by photoinduced electron transfer (PET)-FCS

H. Neuweiler[1], M. Loellmann[2], S. Doose[2], M. Sauer[2]

[1]Medical Research Council Centre for Protein Engineering, Hills Road, Cambridge CB2 2QH, United Kingdom, [2]Applied Laser Physics and Laser Spectroscopy, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

Detailed information about the dynamics of conformational fluctuations of proteins and nucleic acids in aqueous environment is imperative for a refined understanding of biomolecular recognition and folding pathways. We present data about structural changes of single biomolecules, i.e. oligonucleotides, and peptides determined under equilibrium conditions in solution occurring on time scales ranging from nanoseconds to milliseconds. The technique is based on quenching photoinduced electron transfer (PET) reactions between guanosine or tryptophan residues in proximity to selected fluorophores in combination with fast fluorescence correlation spectroscopy (PET-FCS). We demonstrate that PET-FCS can be used advantageously to study fast folding phenomena at the single-molecule level under thermodynamic equilibrium with nanosecond time resolution. Using PET-FCS we monitor folding transitions as well as conformational flexibility in the denatured state of a 20-residue protein (Trp-cage) and different mutants. Besides microsecond folding kinetics we reveal hierarchical folding of Trp-cage, hidden to previous experimental studies. Finally, we use the technique to study the influence of macromolecular crowding on polypeptide chain dynamics. Here our data suggest that within a cellular environment the early formation of structural elements in unfolded proteins can still proceed quite efficiently in spite of hindered diffusion caused by high macromolecular content.

P-491

Purification of the retinal ABCA4 transporter for structural and functional studies

P. H. Niesten[1], C. A. McDevitt[1], R. F. Collins[2], R. C. Ford[2], I. D. Kerr[3], R. Callaghan[1]

[1]Nuffield Dep. of Clinical Lab. Sciences, JR Hospital, Univ.of Oxford, U.K., [2]Faculty of Life Sciences, Univ. of Manchester, U.K., [3]Centre for Biochemistry & Cell Biology, QMC, Nottingham, U.K.

ABCA4 is a member of the ATP binding cassette (ABC) superfamily of membrane transporters, localised to the disk membranes of photoreceptor cells. Mutations in the *ABCA4* gene are responsible for several autosomal recessive retinal degenerative diseases including Stargardt's disease. This suggests a role for ABCA4 in the visual cycle, most likely in the transport of a retinal derivative. Several substrates for ABCA4 have been proposed, including all-trans-retinal and the lipid conjugate, *N*-retinylidene-PE. The aims of our investigations are (i) characterise whether these compounds interact with ABCA4 and (ii) describe the structure of ABCA4 using electron microscopy (EM). The protein was engineered with a C-terminal His_{12} tag and expressed in insect cells using recombinant baculovirus. Unfortunately, non-ionic detergents could not extract more than about 5% of the ABCA4 protein. Only the detergents foscholine-14 and -16 were able to extract ABCA4 from insect cell membranes. The soluble protein was subjected to Ni-NTA chromatography and subsequent size exclusion chromatography, and a purity of more than 90% was achieved. The protein-detergent complex had an apparent molecular mass of 1075 kDa as compared to soluble molecular mass standards. The sample was detergent exchanged into dodecyl-β-maltoside to enable EM studies of the protein. Single particle analysis of the protein is now underway. Furthermore, the reconstitution of the purified protein into lipid vesicles is being undertaken using a detergent adsorption technique.

Abstracts

– Single molecule imaging and spectroscopy –

P-492

Rates of contact formation in disordered proteins: simulation and theory vs experiment

E. Paci[1], M. J. Feige[2]
[1]University of Leeds, UK, [2]TU Munich, Germany

Experimental techniques with high temporal and spatial resolution have made a convergence between simulation and experiment possible. In particular, triplet-triplet energy transfer (TTET) reveals the formation of contacts within peptides with ps time resolution. Such advances in the experimental techniques allow us to directly compare theoretical, simulation and empirical results. Such comparison provides a microscopic interpretation of experimental rates, and reveals deficiencies of force-fields and the utility of theoretical models in explaining the properties of disordered proteins.

P-493

Development of BiFL system for single-pair FRET to analyse interacting protein populations

S. Pathmananthan, S. M. Ameer-Beg, H. K. Tony Ng
Division of Cancer Research & Randall Division of Cell and Molecular Biophysics, Richard Dimbleby Dept. of Cancer Research, King´s College London, UK

Measurement of the near-field localisation of protein complexes may be achieved by the detection of Förster resonant energy transfer (FRET) between protein-conjugated fluorophores. Even when coupled to multiphoton-based, high-resolution fluorescence lifetime imaging (FLIM), these ensemble FRET-based assays only allow us to describe the 'average' behaviour of the interacting proteins and do not allow fluorophore separation distances to be accurately calculated, as is possible with single molecule-derived or single-pair (sp) FRET. Fluorescence detection of single molecules represents the ultimate in sensitivity for fluorescence-based assays in biology and medicine. We describe the development of a time-resolved multiphoton methodology to observe single-molecule dynamics and measure fluorescence lifetime as they diffuse through a femtolitre volume. Streptavidin labelled quantum dots and biotin labelled Alexa Fluor 546 served as the FRET pair. By applying complementary techniques such as fluorescence correlation spectroscopy (FCS) and burst integrated fluorescence lifetime (BiFL), we obtain both ensemble properties of the bimolecular system (molecular diffusion) in addition to lifetime distributions. Variation of the acceptor concentration and the resulting changes in fluorescence lifetimes and other parameters will be presented. This study seeks to validate fluorescence lifetime based sp-FRET as a quantitative method to investigate the function of protein interactions for validation of novel biomarkers.

O-494

Nanopipette dosing for triggered single molecule experiments

J. D. Piper[1], Y. E. Korchev[2], L. Ying[3], D. Klenerman[1]
[1]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK, [2]MRC Clinical Science Centre, Imperial College London, London W12 ONN, UK, [3]Biological Nanoscience, National Heart and Lung Institute, Imperial College London, London SW7 2AZ, UK

There is increasing interest in studying molecules in non-equilibrium conditions to initiate important biological processes such as folding or catalysis. However currently complex microfluidics are required for single molecule sample preparation and the introduction of analytes to specific areas. Here we present a simple and general alternative that is based on the use of nanopipettes. This is able to locally dose with a precision of a few microns by using distance feedback control based on ion current. Using Total Internal Reflectance Fluorescence (TIRF) imaging, we have performed quantitative analysis of Na^+ dosing to characterise the source as steady state diffusion. Extremely confined pH changes are also possible and this has allowed buffering effects to be investigated using fluorescein. Variation in dosing voltage and pipette size enable a wide range of concentration and pH regimes to be repetitively accessed over 1 to 60 μm areas. This is especially useful for triggering individual molecules without dosing the whole sample surface. In addition the integration with scanning ion conductance microscopy (SICM) provides the capability to locally deliver over live cells.
Ref: Ying, L. M. et al., Phys. Chem. Chem. Phys. (2005), 7, 2859-2866; Piper, J. D. et al., JACS (2006), 128, 16462-16463.

P-495

Investigation of apoptosis regulation in mitochondrial membrane: from model to *in vivo* studies

M. A. Sani[1], E. J. Dufourc[2], G. Gröbner[1]
[1]Department of Chemistry, Umeå University, Sweden, [2]IECB, UMR 5248 CNRS-Université Bordeaux 1, France

One major pathway of programmed cell death is regulated by an imbalance between pro- and anti-apoptotic proteins of the Bcl-family that act at the mitochondrion level. In order to elucidate the malfunction of the process *e.g.* in cancer, we study the impact of the BH4 domain of the pro-survival Bcl-2 protein on mitochondrial membranes, since this interaction seems to be essential to block any apoptotic activation. Two different lipid systems for mitochondrial membranes have been used: neutral and negatively charged vesicles mimicking the outer membrane (POPC/POPE) and contact sites (POPC/POPE/Cardiolipin), respectively, the later being involved in stabilisation of mitochondrial membrane proteins. Our first results reveal that the BH4 domain requires cardiolipin for conversion into α-helix structure. In contact with neutral membranes, the peptide aggregates as β-sheets on the surface. By using high resolution solid state ^{31}P MAS NMR, one could resolve for each lipid component separately the impact of the peptide. In addition, the BH4 domain decreases the lipid cooperativity and increases the aliphatic chain order of the phospholipids as revealed by ^{2}H NMR. Beside the use of model membranes, to establish a relevant model on apoptosis regulation, we decided to monitor the BH4/mitochondria interactions at a molecular level with *in vivo* mitochondria. In order to investigate this challenge, different solid state ^{31}P NMR techniques were applied, with RAMP-CP MAS experiments the most promising ones to monitor only phospholipid fractions with reduced dynamics due to the presence of strong lipid-peptide interactions.

Abstracts

– Single molecule imaging and spectroscopy –

P-496

Resolving single kinesin motors in motion by atomic force microscopy

I. A. T. Schaap[1], C. Carrasco[2], P. J. de Pablo[2], C. F. Schmidt[3]

[1]National Institute for Medical Research, Mill Hill, London, UK, [2]Universidad Autonoma de Madrid, Madrid, Spain, [3]Georg-August-Universität, Göttingen, Germany

Kinesins are dimeric motor proteins involved in intracellular transport along microtubules in eukaryotic cells. Single motor proteins of the Kinesin-1 family can move along microtubules by taking hundreds of successive steps. How exactly a single motor proceeds and how dense traffic is accommodated and regulated on the 13 narrow "lanes" or protofilaments of a microtubule remains unknown because the required resolution lies beyond the reach of light microscopy. We have recently succeeded to follow single Kinesin-1 dimers in their motion along microtubules with nanometre resolution by atomic force microscopy. We found that both heads of one Kinesin-1 dimer are bound for the major part of the chemical cycle time to the microtubule. Furthermore, we could resolve that both heads bind to the same protofilament, instead of straddling two, and remain on this track during processive movement.

P-497

Malaria parasite actin filament structure

S. Schmitz, S. N. Harder, I. A. T. Schaap, M. Grainger, L. J. Calder, J. Kleinjung, P. B. Rosenthal, A. A. Holder, C. Veigel

National Institute for Medical Research, London NW7 1AA, United Kingdom

A novel form of acto-myosin regulation has recently been proposed in which the polymerisation of new actin filaments regulates apicomplexan motility by providing the track for myosin.[1] Both tissue-purified and expressed parasite actins show unusual filament dynamics, the structural details of which remain unknown.[2,3,4] Here we extracted as G-actin almost all of the actin present in the merozoites of *Plasmodium falciparum*. Using the F-actin-stabilising toxin jasplakinolide (JAS), >90% of the extracted actin could be polymerised. Malaria and rabbit skeletal (+/-JAS) actin filaments were imaged by negative stain electron and atomic force microscopy. Parasite actin filaments were significantly longer than those observed previously in the absence of JAS.[2] Both the genetic and double helix of actin could be resolved. Image processing revealed structural differences between malaria and rabbit skeletal actin filaments. These differences may be critical for the different filament dynamics observed and might be crucial for parasite motility.

1. Wetzel D *et al*. (2003) Mol Biol Cell 14, 396-406.
2. Schmitz S *et al*. (2005) J Mol Biol 349, 113-125.
3. Schüler H *et al*. (2005) Mol Biol Cell 16, 4013-4023.
4. Sahoo N *et al*. (2006) Mol Biol Cell 17, 895-906.

P-498

Interacting triplet states initiate ion-radical decomposition of adenosinetriphosphate

A. A. Tulub, V. E. Stefanov

Saint-Petersburg state university, Saint-Petersburg, Russian Federation

Unrestricted configuration interaction (2x10 (6) configurations, 6–31G** basis set, T = 310 K) quantum chemistry method is used to study interactions between adenosinetriphosphate (ATP), ATP subsystem, and $[Mg(H2O)_6]^{2+}$, Mg subsystem, in their triplet (T) and singlet (S) states in water environment, modeled with 78 water molecules. T potential energy surfaces, PESs, lie above S PESs and, unlike them, direct the Mg complex towards B-A phosphate oxygens, O2–O3 (S PESs direct the complex towards A-B phosphate oxygens, O1–O2). On its way to O1–O2 and O2–O3, Mg complex loses two and four water molecules, respectively. T PESs reveal a crossing in the vicinity of O2, r[Mg–O2] = 2.19 Å, where redistribution and reorientation of spins occurs. This results in appearing three differing in energy states: $T12^*$, S^*, and R. $T12^*$ state is the highest in energy and no more than a virtual. S^* state, corresponding to spin reorientation in such a way that total spin appears to be of S symmetry, directs Mg subsystem further to form O2–O3 chelate. R state of total S symmetry suggests that spins are localized within each subsystem and form a weakly coupled radical pair. This pair is unstable and rapidly decomposes, yielding ADP(2-) (adenosinediphosphate ion-radical) or AMP(-) (adenosinemonophosphate ion-radical), depending on the extent to which the reaction proceeds. Ion-radical production is crucial in understanding the nature of polymerization reactions with ATP and other nucleosidetriphosphates.

The research is supported by the Grant RNP 2.1.1.4139.

P-499

Mobility of GPCRs reveals a new level of control in directional sensing during migration

F. van Hemert[1], S. de Keijzer[3], A. Pezzarossa[1], C. Aleman[1], B. E. Snaar-Jagalska[2], T. Schmidt[1]

[1]Leiden Institute of Physics, The Netherlands, [2]Institute Biology Leiden, The Netherlands, [3]Dept. of Electrical and Computer Engineering, The Johns Hopkins University, Baltimore, U.S.A.

Chemotaxis involves three complex and interrelated processes: directional sensing, cell polarization and motility. Directional sensing allows highly migrating eukaryotic cells to chemotax in extremely shallow (<2%) gradients of the chemoattractant. Although directional sensing has been observed as spatially restricted responses along the plasmamembrane, our basic understanding of how cells process the gradient-controlled translocation of proteins during chemotactic movements is still largely lacking. Until now, the dynamics of the chemoattractant-receptor has been neglected in models describing directional sensing mechanisms. Here we show by single-molecule microscopy an agonist-induced increase in mobility of cAMP-receptor-eYFP at the leading edge of chemotacting *Dictyostelium* cells. Furthermore, we found this mobility shift is linked to the uncoupling/activation of the Gα2-protein. An in silico model confirmed that a single activated/mobile receptor can activate multiple G-proteins thereby providing a mechanistic basis for a primary amplification step at the level of the G-proteins in current theoretical models describing directional sensing.

Abstracts

– Single molecule imaging and spectroscopy –

P-500

Mapping nano-landscape of pathogen recognition receptor DC-SIGN and lipid rafts on dendritic cells

T. van Zanten[1], J. Zambrano[1], M. Koopman[2], A. Cambi[3], B. Joosten[3], C. G. Figdor[3], M. F. García-Parajó[1]

[1]Bionanophotonics group; IBEC, Barcelona, Spain, [2]Optical Techniques group; University of Twente, Enschede, The Netherlands, [3]Tumor Immunology; NCMLS, Nijmegen, The Netherlands

The dendritic cell (DC) specific pathogen-recognition receptor DC-SIGN binds and internalizes antigens for degradation. The organization of DC-SIGN in microdomains is crucial for the binding and the internalization of virus particles, suggesting that these multimolecular assemblies act as docking site for pathogens like HIV-1 to invade the host. We have recently shown that DC-SIGN potentially associates with lipid rafts [1] and clathrin coated pits [2]. Nevertheless, the nano-scale organization of DC-SIGN with respect to these lipid domains remains largely unresolved. To map the nano-landscape distribution of DC-SIGN on DC cell membranes we are exploiting state-of-the-art microscopic imaging techniques. Single fluorescent molecule detection together with multicolor labeling offers the possibility to elucidate organization and co-localization at <100 nm spatial resolution. Currently we are investigating the potential association of DC-SIGN with lipid rafts using a near-field optical microscope working under physiological conditions. Additionally we are planning a three color experiment to resolve the nano-landscape of DC-SIGN, lipid rafts and clathrin coated pits before and during endocytosis. The possible change in organization and association of DC-SIGN is essential for the understanding of the antigen uptake mechanism(s) from the membrane of DCs. [1] A. Cambi, et al., *Journal of Cell Biology*, **164**, 145 (2004). [2] A. Cambi, et al., *Nanoletters*, In Press.

P-501

The crystal structure of CobE, a protein involved in cobalamin (vitamin B12) biosynthesis

J. Vevodova[1], D. Smith[2], H. McGoldrick[3], E. Deery[3], M. J. Warren[3], K. S. Wilson[1]

[1]Structural Biology Laboratory, Department of Chemistry, University of York, U.K., [2]Cheminformatics Group, Bioinformatics Institute, Singapore, [3]Protein Science Group, Department of Biosciences, University of Kent, U.K.

Vitamin B_{12} (cobalamin) is a modified tetrapyrrole that belongs to the family of metallo-prosthetic groups. The structural complexity of vitamin B_{12} is reflected in its biosynthesis requiring around 30 enzymes for the complete *de novo* construction of the coenzyme form. For cobalamin biosynthesis two distinct yet similar routes exist, aerobic and anaerobic pathways. The aerobic pathway is found in *Pseudomonas aeruginosa.* The enzymes of the aerobic pathway are given the prefix Cob. In the aerobic pathway there are some proteins that are known to be required for cobalamin biosynthesis yet for which no function has been assigned. One of these is CobE, a comparatively small protein with a molecular mass of about 15 kDa. The crystal structure of the protein CobE has been solved at a resolution of 1.9 Å, and reveals a novel fold. CobE is a protein that has been previously been identified as an essential component of the aerobic cobalamin biosynthetic pathway although its precise role has not been elucidated. The protein shares similarity with part of CbiG, an enzyme that is normally found associated with the anaerobic cobalamin biosynthetic pathway, and which has recently been shown to be involved in lactone ring opening and C_2 extrusion during corrin ring formation. The possibility exists therefore that CobE plays an equivalent role in the aerobic pathway.

P-502

Intrinsic motions along an enzymatic reaction trajectory

M. Wunderlich[1], K. Henzler-Wildman[2], D. Kern[2], C. G. Hübner[3]

[1]Institute of Physics, Martin Luther-University Halle-Wittenberg, Hoher Weg 8, D-06120 Halle, Germany, [2]Department of Biochemistry and Howard Hughes Medical Institute, Brandeis University, Waltham, MA 02454, [3]Institute of Physics, University at Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany

Enzyme kinetics involves conformational changes of the substrate/enzyme complex. While chemical mechanisms have been elucidated for many enzymes, the question of how enzymes achieve the catalytically competent state has only recently become approachable by experiment and computation.

We report on single pair FRET experiments on the two-substrate enzyme adenylate kinase. Intrinsic motions of the open and closed conformations of inhibitor- and substrate-bound adenylate kinase were found to occur on a microsecond to millisecond time scale. Furthermore, the experiments reveal the rare sampling of a fully closed conformation of the substrate-free adenylate kinase which indicates that the larger-scale motions in substrate-free adenylate kinase are not random but preferentially follow the pathways that create the configuration capable of proficient chemistry.

P-503

Elimination of adsorptive behaviour of biomolecules at the glass-solution interface in fluorescence correlation spectroscopy

D. Zhang, R. Vermeij, A. Lenferink, I. Segers-Nolten, C. Blum, V. Subramaniam, C. Otto

MESA+ Institute for Nanotechnology, Biophysical Engineering Group, University of Twente, Netherlands

We study adsorptive behaviour of biomolecules at the glass-solution interface in fluorescence correlation spectroscopy (FCS), and propose a negatively charged coating to eliminate the adsorption of molecules. In this article, we demonstrate confocal microscopic measurements on Cy3.5-90-mer-ssDNA and Cy3.5-90-bp-dsDNA in different solutions, and use two polymers – poly (acrylic acid, sodium salt) and poly (sodium 4-styrenesulfonate) to produce the negatively charged coating on glass coverslips. This technology enables more stable FCS measurements in extremely low concentration samples and reveals that the adsorptive behaviour of biomolecules is responsible for sudden disappearance of many biomolecules in low concentration solutions.

Abstracts

– Single molecule imaging and spectroscopy –

P-504

Single molecule analysis of Lck-movement in Jurkat T cells

L. Zimmermann[1], J. Weghuber[1], W. Paster[2], H. Stockinger[2], G. J. Schütz[1]

[1]Institute for Biophysics, Johannes Kepler University Linz, Austria, [2]Institute of Immunology, Medical University of Vienna, Austria

Lck is a major Src-family kinase in T-cell signaling, which regulates signal transduction by specific phosphorylation of ITAMs on the T cell receptor complex. We investigated the dynamic behaviour of Lck in Jurkat T cells using total TIRF-microscopy. Analyzing the diffusion of Lck, labeled with YFP, revealed two fractions, one third of the Lck molecules are immobile (D = 0.02 $\mu m^2/s$), the other fraction is mobile (D = 0.6 $\mu m^2/s$). The short duration of single molecle trajectories was indicative of a limited lifetime of Lck in the plasma membrane. Therefore we studied the trajectory length as a function of the laserpower to discriminate the power-independent off-rate from power-dependent contributions like photobleaching. The measured lifetime of Lck-YFP in the plasma membrane of Jurkat T was $\sim$100ms. A caveat of the above assay is its susceptibility to power-independent photophysics, e.g. dye blinking. We therefore controlled the measurements on YFP by a novel intracellular labeling technique, using AGT (O6-alkylguanine-DNA alkyltransferase) and tetra-methylrhodamine. TMR is advantageous compared to YFP due to its higher photostability and reduced blinking. Using TMR as a fluorescent label allowed us to measure trajectory lenghts longer than 80 frames. Lck-AGT-TMR was found to reside in the plasma membrane up to several seconds. The discrepancy with the results obtained on Lck-YFP clearly reveals the potential bias of power variation for off-rate analysis, and indicates the need for control experiments using different labels.

Abstracts

– Membrane microdomains & signalling –

P-505

Molecular crowding of DnaA on the membrane surface – a switch for the nucleotide exchange

A. Aranovich[1], G. Y. Gdalevsky[2], R. Cohen-Luria[2], A. H. Parola[2], I. Fishov[1]

[1]Life Sciences, Ben-Gurion Univ of the Negev, Beer-Sheva, Israel, [2]Chemistry, Ben-Gurion Univ of the Negev, Beer-Sheva, Israel

The activity cycle of DnaA, the initiator protein for chromosomal replication in bacteria, is driven by ATP binding and hydrolysis that couples key events during the initiation. The membrane containing acidic unsaturated phospholipids promotes the reactivation of the DnaA activity by accelerating the nucleotide exchange. We examined how the kinetics of membrane-induced nucleotide dissociation from DnaA, followed by the fluorescent analog of ATP, MANT-ATP, depends on the phospholipid/protein ratio. The results reveal that macromolecular crowding on the membrane surface accounts for an unusual, highly cooperative transformation from a relatively slow to a high rate of nucleotide exchange on membrane-bound DnaA at varying protein surface densities. Distinct temperature dependences and the effect of the crowding agent ficoll corroborate two functional states of DnaA at high and low membrane occupancy. DnaA lacking the domain responsible for DnaA-DnaA interaction lost the ability for the cooperative activation, suggesting that the DnaA oligomerization could be a prerequisite to the crowding-dependent activity transformation. We propose that the specific membrane domain, unique in its acidic phospholipid composition, serves as a catalyst in activating DnaA at the right time, which is when the size of the continuously growing bacterial membrane domain during the cell cycle reaches a critical DnaA-to-phospholipid ratio, switching on (due to cooperativity) the reactivation of DnaA, which in turn initiates replication.

P-506

Shiga Toxin induces tubular membrane invaginations

L. Berland[1], W. Römer[2], P. Sens[3], L. Johannes[2], P. Bassereau[1]

[1]Laboratoire Menbranes et Fonctions Cellulaires, UMR 168, Institut Curie, Paris, France, [2]Laboratoire Trafic Signalisation, UMR 144, Institut Curie, Paris, France, [3]Physico-Chimie Théorique, CNRS UMR 7083, ESPCI, Paris, France

Many extracellular molecules enter cells via endocytic routes that do not involve clathrin-coated pits. A good example is the bacterial Shiga toxin (STxB) that binds to Gb3 glycolipids.

In conditions where most of active uptake processes are blocked (ATP depletion and low temperature), our collaborators have shown that STxB is still internalized by cells and found in tubular structures.

Using a minimal model system (Giant Unilamellar Vesicles containing Gb3, and purified proteins), we mimic formation of similar tubular structure, demonstrating that STxB and lipid membrane are the minimal components to form tubular cell intermediates. We have investigated the role of relevant physical parameters such as membrane tension, lipid organization or lipid geometry to unveil mechanisms of this cargo-induced membrane invaginations.

Further results obtained with cholera toxin or viral capsids show that we are in presence of a more general process of uptake.

P-507

Ternary mixture of sphingomyelin, POPC, and cholesterol - microdomains at high temperatures

A. Bunge[1], P. Müller[2], M. Stöckl[2], A. Herrmann[2], D. Huster[1]

[1]Institute of Biotechnology, MLU Halle-Wittenberg, [2]Institute of Biology/Biophysics, Humboldt-University Berlin

Ternary mixtures of sphingomyelin, unsaturated phosphaditylcholines, and cholesterol represent a model system for the outer leaflet of eukaryotic cell membranes, characterized by lateral lipid domains, called rafts. While the formation of lateral domains in lipid membranes at low temperature has been well established, it remains open whether domains also exist at physiologically more relevant temperatures where no segregation can be detected by optical methods. Further, it is unclear how the distribution of cholesterol in the ternary mixture changes with temperature.

In this study, the ternary mixture of palmitoyl-sphingomyelin (PSM), palmitoyl-oleoyl-phosphatidylcholine (POPC), and cholesterol was studied. Upon decrease in temperature, a redistribution of cholesterol into liquid ordered PSM/cholesterol domains and depletion of the sterol from liquid disordered POPC domains was observed by ^{2}H NMR and EPR experiments as well as confocal fluorescence microscopy. However, there is no complete segregation of cholesterol into the liquid ordered phase and also POPC rich domains contain the sterol in the phase coexistence region. We further compared order parameters and packing properties of deuterated PSM or POPC in binary and raft mixtures at temperatures above the main phase transition of the two phospholipids. The results can be explained by an inhomogeneous distribution of cholesterol between the two lipids and the mutual influence of the phospholipids on each other. At high temperatures, the raft mixture shows a maximum microdomain size of 35-60 nm.

P-508

Signal transduction and ligand discrimination by dynamic spatial relocation to microdomains

N. J. Burroughs[1], A. P. van der Merwe[2]

[1]Warwick Systems Biology Center, University of Warwick, U.K., [2]Sir William Dunn School of Pathology, Oxford University, U.K.

Many receptors appear to have membrane signalling mechanisms that do not involve conformational change or oligomerisation, in particular immunological receptors such as the T cell receptor (TCR), B cell receptor and Fc receptor. It has been proposed, in the so called *kinetic segregation hypothesis*, that these receptors function through relocation to phosphatase depleted microdomains, ligand binding driving this relocation. We develop a model of tyrosine phosphorylation by localization to regions of close membrane-membrane proximity (close contact) that physically exclude large tyrosine phosphatases such as CD45, an exclusion that generates regions of low phosphatase and high kinase activity. The residence time of unbound receptors in tyrosine kinase-rich domains is shown to be too short for accumulation of activation steps, whereas binding to an agonist lengthens the localization time and leads to generation of fully active receptors. Ligand detection depends only on this localization, and therefore kinetic segregation represents a specific and sensitive viable ligand detection mechanism distinct from receptor oligomerization and conformational change.

Abstracts

– Membrane microdomains & signalling –

O-509

Membrane protein organization and ceramide domains: a combined AFM and FCS study

S. Chiantia, N. Kahya, J. Ries, P. Schwille
Biotec Zentrum, Biophysics group, TU Dresden, Germany

A combination of fluorescence correlation spectroscopy (FCS), laser scanning microscopy and atomic force microscopy (AFM) has been used to investigate supported bilayers composed of sphingomyelin (SM), dioleoylphosphatidylcholine (DOPC), cholesterol and ceramide (Cer). This lipid composition is known to produce a liquid-disordered membrane containing both liquid-ordered "raft-like" domains and Cer-enriched domains. Cer-induced alterations of microdomains and membrane protein distribution in cell membranes are involved in several biological processes including apoptosis, immune response and viral or bacterial infections. We studied the effects of Cer with different chain lengths on the lateral organization of the membrane. Furthermore, we reconstituted membrane proteins with different characteristics in the model bilayer, i.e. GPI-anchored alkaline phosphatase (PLAP) and synaptobrevin, in order to investigate their lateral organization in the presence of mixed raft-like and Cer-enriched domains. Our results reveal, on a nanoscopic scale, the structural features of the lipid bilayer and the spatial organization of the embedded proteins monitored by AFM. Furthermore, FCS is used to probe the diffusion and the partition of proteins and lipids in the distinct lipid phases. In conclusion, we show that long-chain Cer induce the formation of a lipid phase characterized by high structural and diffusional order. These Cer-enriched domains exclude the majority of the membrane components (like synaptobrevin and fluorescent lipid analogues) but, surprisingly, are rich in molecules known as "raft-associated", like ganglioside-bound cholera toxin and PLAP.

O-510

Dynamic map of the plasma membrane organization by FCS & Multiple Target Tracing analyses

F. Conchonaud[1], A. Sergé[1], N. Bertaux[2], P.-F. Lenne[2], A. Boned[1], H. Rigneault[2], H.-T. He[1], D. Marguet[1]
[1]Centre d´Immunologie de Marseille Luminy - CNRS - INSERM, Marseille, France, [2]Institut Fresnel - CNRS, Marseille, France

We have established an original and robust fluorescence correlation spectroscopy (FCS) approach to investigate the dynamic molecular confinement in the plasma membrane of living cells. By performing FCS measurements on various observation scales, this method is sensitive to nanoscale confining structures allowing us to investigate the complexity of the cell membrane organization [1]. The diffusion behavior of various membrane components in living cells was described and it was established whether molecular confinement depends on the specific lipid membrane content or on the presence of actin-based cytoskeleton barriers. More recently, we have provided significant observations supporting that, *in vivo*, the association of specific proteins with these nanodomains is required for the efficient recruitment/signal transduction functions associated with these molecules [2]. To fulfill these FCS analyses, we have develop a new powerful approach to draw a dynamic map of confining and fast diffusing areas present at the plasma membrane. This approach is based on a new Multiple Target Tracing algorithm by characterizing local diffusion coefficient from a high number of single molecule trajectories observed over a short period of time. Using such approach, we provide a snapshot of the membrane dynamics.
[1] **Biophys J** 89:4029; **EMBO J**. 25:3245; **Biophys J** 92:913
[2] **Blood** 107:2341; **EMBO J** 26:209

P-511

Modulation of leucocyte plasma membrane microdomains by heparan sulfate

J. Duggan, B. Davis, E. Horn, K.-A. Vere, P. O'Shea
Cell Biophysics Group, Institute of Biophysics, Imaging and Optical Sciences, University of Nottingham, UK

Membrane microdomains composed predominantly of sphingomyelin and cholesterol represents phase separated regions of the plasma membrane that have been implicated in a wide variety of events from HIV infection, malarial infection and prion diseases through to cellular events such as exocytosis, endocytosis and cell signalling. Despite this, the mechanism regulating the formation and deformation of membrane rafts is still unknown.
Heparan Sulfate (HS) is a highly negatively charged polysaccharide found ubiquitously at the cell surface in the form of proteoglycans. The structure is based upon a repeating disaccharide but which undergoes considerable modifications, resulting in interspersed non-sulfated and sulfated domains. The later of which is responsible for the binding of chemokines and other extracellular ligands that HS is known to bind acting as a molecular net, presenting ligands to receptors on the cell surface.
We report results consistent with the disassembly of membrane rafts as a result of the addition of free HS or through treatment of the cells with Heparinase III. The disassembly of the tightly packed rafts is accompanied by a subsequent increase in cell size, measured with light scattering techniques and visualised utilising confocal microscopy. We suggest a model of where the HS present on the cell surface as part of the proteoglycan syndecan-4 interacts with the FGF receptor in order to stabilise coalesced rafts. The addition of free HS or treatment with heparinase disrupts this system and leads to their deformation.

P-512

Reversibility of polycation interaction with anionic liposomes

A. A. Efimova, A. V. Sybachin, A. A. Yaroslavov
Polymer Department, Faculty of Chemistry, Moscow State University, Moscow, Russian Federation

Interaction of synthetic polycation poly(N-ethyl-4-vinylpyridinium) (PEVP) with small liposomes, composed of negatively charged cardiolipin (CL) and neutral dipalmitoylphosphatidylcholine (DPPC), has been investigated.
Binding of PEVP to liquid liposomes (the fluid state of the membrane) leads to significant structural rearrangements in the membrane: lateral lipid segregation, flip-flop of CL molecules, etc. However, the integrity of the membrane retains unchanged. The interaction is electrostatic in nature: the polycation is completely removed from the membrane by polyacid addition or when increasing salt concentration. The removal of polycation restores the initial distribution of lipids in the lateral and transmembrane directions.
In contrast to that, adsorption of PEVP on the surface of solid liposomes (the gel state of the membrane) produces defects in the liposomal membranes. This makes the complexation irreversible: PEVP keeps contacting solid liposomes in the presence of abundant amount of PAA-anions or when increasing salt concentration.
Importantly, the defects remain when transferring solid liposomes, covered by PEVP, to the liquid. Moreover, the reversible contact of PEVP with liquid liposomes becomes irreversible as PEVP-bound liposomal membranes are converted to the gel state. The dependence of properties of polycation-liposome complexes on the phase state of the lipid membrane was demonstrated.

Abstracts

– Membrane microdomains & signalling –

P-513

The effect of divalent counterions in phosphatidic acid domains in membranes: computer simulations.

J. Faraudo[1], A. Travesset[2]

[1]Institut de Ciencia de Materials de Barcelona (ICMAB-CSIC) and Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Ames Laboratory and Dept. of Physics and Astronomy, Iowa State University, Ames, Iowa, U.S.A.

Phosphatidic acid (PA) is emerging as a key phospholipid in a wide range of biological processes such as signal transduction, secretion, or membrane fusion. In most cases, the biological functionality of PA is associated with the presence of micromolar to millimolar concentrations of divalent counterions such as Ca. It has been argued that PA can create defects in the packing of lipids in membranes due to lateral phase separation by divalent ions, which in turn aggregate proteins with high affinity for PA. In this work, we present a detailed investigation of the properties of PA domains in the presence of divalent ions by a combination of molecular dynamics simulations and theoretical methods. Our results show that PA is extremely effective in binding divalent ions through its oxygen atoms. We predict that a PA-rich domain undergoes a drastic reorganization when divalent cations reach micromolar concentrations(i.e., typical physiological conditions), exhibiting the phenomenon of charge inversion (a total number of bound counterion charges that exceeds the negative PA charge). We also discuss the properties of interfacial water, which are relevant in the binding of proteins or other molecules to the membrane. We conclude with a discussion of the implications of our results in the context of recent experimental studies in model systems and in real cells.

P-514

Update on biological physics in the Physical Review and Physical Review Letters

M. C. Foster

Physical Review Editorial Office, Ridge, 11961 New York, U.S.A.

Physical Review E and Physical Review Letters have growing sections devoted to biological physics. Both PRE and PRL are indexed in MEDLINE. Recent innovations for the journals include information about citations in other journals. In January 2007, PRL began including in each issue Editors' Suggestions of a small number of Letters that may be of particular interest to readers outside their fields of expertise. Within the past year, open access, or "Free to Read," has allowed individuals or institutions to pay a modest fee in order to make the full text of selected articles available to readers without subscriptions. Authors and referees come from the international community, with a large number from European countries. Papers are published in a wide variety of biologically inspired physics topics, including biological molecules, cellular and subcellular systems, properties of higher organism, and ecology and evolution. For regular papers in PRE, the median time to receive the first referee report is about 3 weeks and the median time from submission to acceptance is about 100 days. The acceptance rate is approximately 60% for PRE and 35% for PRL. Authors of accepted papers may suggest their papers for publicity and outreach to the public. The impact factor is 2.4 for PRE and 7.5 for PRL. We continue to welcome suggestions for ways to improve the value of the journals to the scientific community.

P-515

Interaction of HIV fusion inhibitor Sifuvirtide with lipid membranes: Fluorescence and AFM approach

H. G. Franquelim[1], N. C. Santos[2], M. A. R. B. Castanho[1]

[1]Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, [2]Unidade de Biopatologia Vascular – Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisboa, Portugal

The interaction of HIV fusion inhibitor Sifuvirtide (a 36 amino acid negatively charged peptide) with biological and non-biological membrane model systems was studied using fluorescence spectroscopy techniques (both steady-state and time-resolved). Intrinsic fluorescence properties of its tryptophan (Trp) residues were used to study its interaction with POPC, POPC/Cholesterol and positively charged POPC/EPOPC large unilammelar vesicles. Results showed no significant interaction of the peptide neither with POPC or POPC/Cholesterol vesicles. However significant partition was observed in the positively charged lipid models (K_p=2,2$\times 10^3$), serving as a positive control. Fluorescence quenching using lipophilic probes and acrilamide was carried out to study the location of the peptide in the membrane models. It was concluded that the Trp residues are located near the interface of the membrane bilayers. Relatively to the DPPC membrane model, a complex interaction of the peptide was observed. These results indicate some kind of scrutinity of the peptide to gel phase membranes. This larger affinity with the more rigid membrane areas may help explain the improved clinical efficiency of Sifuvirtide. To complement the fluorescence results, several lipid bilayer systems and its interaction with Sifuvirtide were imaged using Atomic Force Spectroscopy (AFM).

Acknowledgment: Sifuvirtide was a kind gift from FusoGen Pharmaceuticals.

P-516

Molecular approaches to the study of UV-B induced stress responses in two extremophiles ciliates

L. Fulgentini[1], R. Marangoni[1], V. Passini[2], A. La Terza[2], C. Miceli[2], G. Colombetti[1]

[1]Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Pisa, Italy, [2]Dipartimento di Biologia M.C.A., Università di Camerino, Camerino (MC), Italy

In the last years, release of CFCs caused remarkable changes in the chemistry of the atmosphere, especially regarding ozone. These changes brought variations in spectral distribution for the solar radiation reaching the terrestrial surface, letting more UV-Bs to pass through the stratospheric filter. UV-Bs are the most biologically damaging wavelengths and their absorption by cells can cause severe damage. Even aquatic organisms are not completely safe, albeit they are protected by water which can partially screen these radiations. We focused on the analysis of radiative stress responses in two extremophiles marine ciliates: *F. salina* and *E. focardii*.

Fabrea salina is a halophile ciliate with high resistance to UV-Bs. We studied differential protein expression profile between irradiated and non-irradiated cells of *F. salina* by means of bi-dimensional gel electrophoresis and tried to analyze the differentially expressed proteins by means of mass spectrometry.

Euplotes focardii is an endemic psychrophilic hypotrich ciliate of the Antarctica. We investigated the activation induced by UV-B radiation of the transcription of *hsp70* genes, coding for a family of heat-shock proteins. We analyzed the response to UV in irradiated samples simulating spectral composition and intensity of the solar radiation during Antarctic spring, and varying the total exposure to UV-B radiation. The response was assessed evaluating induced variations on *hsp70* transcripts.

Abstracts

– Membrane microdomains & signalling –

P-517

Edelfosine induces gel phase in a cholesterol-containing liquid ordered membrane.

J. C. Gómez-Fernández, A. Ausili, F. J. Aranda, A. Torrecillas, S. Corbalán-García
Departamento de Bioquímica y Biología Molecular A, Facultad de Veterinaria, Universidad de Murcia. Apartado 4021, E-·30080-Murcia, Spain.

Edelfosine is an ether-phospholipid which is being used as antitumoral. A POPC/sphingomyelin/cholesterol (1:1:1 molar ratio) membrane was studied through ^{2}H-NMR and it showed a transition from liquid ordered to liquid disordered but the addition of 20 mol% of edelfosine induced the appearance of a gel phase at low temperatures with a gel to liquid disordered phase transition at 18°C. In addition, edelfosine increased the order of the membrane below and decreased it above the phase transition. The appearance of the gel phase was confirmed by WAXR-diffraction and DSC. On the other hand, SAXR-diffraction showed that the presence of edelfosine increased the thickness of the membrane and, at 20 mol%, new spacings were detected. In order to clarify the role of cholesterol, we studied samples containing POPC/sphingomyelin (1:1 molar ratio) and this mixture plus 20 mol% edelfosine. DSC and ^{2}H-NMR showed that the effect of edelfosine was to considerably increase the temperature at which the gel to liquid disordered phase transition takes place. This was confirmed by WAXR-diffraction whereas SAXR-diffraction showed a decrease in the thickness of the membrane. ^{31}P-NMR and ^{2}H-NMR showed that the effect of edelfosine was to induce isotropic components corresponding to smaller particles. The conclusion is that edelfosine specifically interacts with cholesterol, and the result is the partial neutralization of the effects of both molecules on membranes

O-518

Membrane microdomains: physics serving biology

P. K. J. Kinnunen
HBBG, Institute of Biomedicine, University of Helsinki, Helsinki, Finland

Biological organisms represent complex systems characterized by coupling between their dynamic, adaptive organization and function. The involved molecular order is due to both equilibrium self-assembly as well as non-equilibrium (dissipative) factors. The paradigm for the above is biological membranes. Regarding the evolution of our understanding of their structure true milestones were the early contributions by J. Israelachvili (1977, 1980) and E. Sackmann (1973) and their collaborators, who depicted the organization of biomembranes into microdomains composed of specific lipids and proteins. These papers triggered efforts in a number of laboratories and using various model membrane systems, yielding our current conception of the functional organization of biomembranes.

Key concepts are phase diagrams for predominant lipid systems, such as comprised of phosphatidylcholine, sphingomyelin, cholesterol, and ceramide, which has turned out to be of importance in determining both 2-D and 3-D organization of membranes. This particular example is described in detail, highlighting the importance of spontaneous curvature and bending rigidity, with examples on how these factors are utilized for instance in the entry of microbes into their eukaryote hosts.

P-519

Regulation of ion transport in colonic epithelium: The role of basolateral chloride channels

K. M. Kosheiff, R. S. Lam, M. Duszyk
University of Alberta, Department of Physiology, Edmonton, Alberta T6G 2H7, Canada

Water and electrolyte absorption is a major function of the colon. A single-cell layer of epithelium lines the colon lumen, and ion channels in the epithelium control transepithelial ion secretion and/or absorption. The presence of Cl^- channels in the basolateral membrane has been established in several studies, but their physiological role is not well characterized. Lipid rafts are cell membrane microdomains thought to be important for several functions of the cell, including cellular signaling. These microdomains are sphingolipid-cholesterol rich structures that house many cell membrane and membrane-associated proteins, including G-proteins, G-protein coupled receptors, and ion channels. Recently several types of ion channels have been shown to have cholesterol-dependent activity and to localize to lipid rafts. In this study, we have investigated the role of cholesterol in the regulation of ion transport. We observed that methyl-β-cyclodextrin (MβCD), a cholesterol-sequestering molecule, activated transepithelial short circuit current, but only from the basolateral side. Similar results were obtained with a cholesterol-binding agent, filipin, and with the sphingomyelin-degrading enzyme, sphingomyelinase. Experiments with ΔF508CFTR mutant mice indicated that raft disruption affected CFTR-mediated anion secretion, while pharmacological studies showed that this effect was due to activation of basolateral Cl^- channels. We propose a new mathematical model of anion secretion in which basolateral Cl^- channels recycle Cl^- across the basolateral membrane, allowing preferential HCO_3^- secretion.

P-520

Phospholipase D activation by calcium induced phase separation

K. Kuppe, R. Ulbrich-Hofmann
Martin-Luther University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, 06120 Halle (Saale), Germany

Phospholipase D (PLD) is a calcium dependent enzyme that catalyzes the hydrolysis of glycerophospholipids to release phosphatidic acid (PA) and the alcohol of the head group. It plays an important role in signalling cascades since PA is a major lipid mediator involved in diverse cellular functions. Interestingly, PA itself is an effector of PLD activity. Here we demonstrate that the incorporation of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate (POPA) into 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) vesicles remarkably stimulates the activity of PLD from cabbage. The rate of POPC hydrolysis was increased up to 500fold and the lag phase was reduced up to 250fold. To analyze whether this stimulation results from a conformational transition into an activated state upon POPA binding to the enzyme or from a specific structural organization of the substrate aggregates, the influence of POPA was studied with soluble substrate and the thermotrophic phase behaviour of POPC/POPA vesicles was studied by Differential Scanning Calorimetry. While direct binding could not account for the large activation by POPA, lateral phase separation within the phospholipid aggregates was observed in the presence of optimum Ca^{2+} concentrations. At the temperature of the enzyme activity assay (25°C) the coexistence of liquid ordered Ca^{2+}-POPA domains within a fluid POPC phase could be detected. As the threshold of POPA proportion for the phase separation is consistent with the threshold of the stimulating effect of POPA on PLD activity, an enzyme regulating role of lipid domains is suggested.

Abstracts

– Membrane microdomains & signalling –

P-521

Role of the membrane interface on the conformation of the caveolin scaffolding domain

C. Le Lan, J.-M. Neumann, N. Jamin
CEA Saclay, Institut de Biologie et de Technologies and URA CNRS 2096, Service de Bioenergétique, Biologie Structurale et Mécanismes, France

Caveolin, a 21 kDa membrane protein is the main protein component of particular microdomains of the plasma membrane (PM) referred as caveolae. In addition to its structural role within caveolae, caveolin is implied in a wide range of cellular functions, especially signalling pathways. The consensus topological model of caveolin includes a small central intra-membrane region (33 aa) flanked by two cytosolic amphiphilic domains. One of these domains named CSD, caveolin scaffolding domain, has been identified as an essential element involved in both membrane anchoring at the interface and interaction with signalling protein. CD and NMR spectroscopy were used to study the conformational properties of two synthetic peptides, D82-R101 and D82-I109, encompassing the CSD sequence, in the presence of DPC micelles. Our data show that the D82-I109 peptide including the L102-I109 segment, a part of the caveolin intra-membrane domain, forms a stable amphiphatic helix in a membrane mimicking system. In a second step, we have studied the interaction between the D82-I109 peptide and phosphatidylserine (PS), an anionic lipid only present in the cytosolic leaflet of the PM. Through chemical shift variations, six residues of the peptide, mainly located in the V_{94}TKYWFYR_{101} motif were found to detect the presence of PS solubilized in micelles. Our results constitute the first direct information at a residue level on the caveolin structure and on its interaction at a membrane interface. (Le Lan C, Neumann, JM, Jamin N (2006). FEBS Lett., **580**, 5301).

P-522

Hydrogen bonding and the sequestering of phosphoinositides by polybasic peptides

C. D. Lorenz[1], J. Faraudo[2], A. Travesset[3]
[1]Materials Research Group, Engineering Division , King´s College London, Strand, London, WC2 2LS, United Kingdom, [2]Department de Fisica, Universitat Autonoma de Barcelona, Bellaterra, Spain, [3]Ames Laboratory and Department of Physics and Astronomy, Iowa State University, Ames, Iowa 50011 USA

Phosphoinositides are a family of phospholipids present in tiny amounts (1% or less) in the cytosolic surface of cell membranes, yet they play an astonishingly rich regulatory role, particularly in signaling processes. In this talk, we use all-atom molecular dynamic simulations to investigate the interaction of Phosphatidylinositol 4,5-bisphosphates, the most common of the phosphoinositides, with a polybasic peptide consisting of 13 lysines. Our results show that the polybasic peptide sequesters three Phosphatidylinositol 4,5-bisphosphates but does not sequester other charged phospholipids such as phosphatidylserine. We provide a detailed account on the sequestering mechanism and discuss the implications of our results for the rapidly increasing number of proteins containing polybasic residues that have been shown to interact with Phosphatidylinositol 4,5-bisphosphate.

O-523

Estimation of rafts size and dynamics from single molecule confocal microscopy of a new membrane dye

A. Margineanu, S. Melnikov, J.-I. Hotta, M. van der Auweraer, F. C. de Schryver, J. Hofkens
Catholic University Leuven, Dept. Chemistry, Lab. Photochemistry and Spectroscopy, Leuven, Belgium

A method for determining the size of the nm-scale rafts (similar to those proposed to exist in resting cells) in confocal fluorescence microscopy with single molecule detection is developed. The spatial information (i.e. rafts size) is obtained from the temporal scale of the exchange dynamics of a fluorescent probe between the liquid disordered (Ld) and the liquid ordered (Lo) domains and from the temporal scale of the diffusion processes in the two phases. The presence of the perylene monoimide dye in the Ld or in the Lo phase can be assigned due to its different fluorescence decay times in the two phases. The time scale of the fluorescence decay time fluctuations (i.e. the exchange dynamics) is determined from the autocorrelation function of photon arrival times (Yang and Xie, J. Chem. Phys., **117**: 10965-79, 2002). By fitting the autocorrelation function with an exponential decay, the average time spent by the fluorescent molecule in a given phase is found. The diffusion coefficients in each phase are obtained from the fluctuations of the fluorescence intensity. The time spent by the dye in a Lo domain and the characteristic diffusion coefficient for the Lo domain are used to calculate the mean square displacement, which can be approximated with the raft size. In order to validate the method and to establish the limits within which the method can be applied, the dye exchange is modeled as a Markov process between two states with two different decay times, and the transition rate constants between the two states are varied.

P-524

Protein clusters in intact membranes and blebs of T lymphoma cells

G. Mocsár[1], A. Bodnár[1], R. Szabó[1], G. Vereb[2], S. Damjanovich[1], G. Vámosi[1]
[1]Cell Biology and Signaling Research Group of the Hungarian Academy of Sciences, Debrecen, Hungary, [2]Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen, Hungary

Major histocompatibility class I and II glycoproteins and interleukin-2 and -15 receptors, which play important roles in immune processes, form supramolecular clusters in FT7.10 T lymphoma cells. Beyond the small-scale molecular associates revealed by FRET, they also form larger aggregates, which are enriched in lipid rafts. We were interested whether the higher order organization of the cell membrane and the cytoskeleton were necessary for maintaining protein-protein interactions. As model system we used apoptotic/necrotic blebs, which do not possess cytoskeletal connections, and there is no sign of any higher order (microdomain) organization at the resolution of confocal microscopy. Whereas MHC molecules, IL-2R and IL-15R formed largely overlapping patches in the intact membrane, they were evenly distributed in blebs, and their mobility increased as measured by FCS. The homoassociation of MHC I, MHC II, IL-2Rα and IL-15Rα as well as the heteroassociation of MHC I with IL-2Rα and IL-15Rα were determined by a confocal microscopic FRET method. FRET efficiencies were equal within experimental error in intact membranes and blebs, i.e. small scale clusters remained stable. The average cluster size revealed by FCS was $\sim$3 for MHC I and II. Our results suggest that interactions stabilizing the studied small-scale protein clusters persist after the disappearance of the higher order organization of the membrane, and do not depend critically on the cytoskeleton.

Abstracts

– Membrane microdomains & signalling –

P-525

Pulsed electromagnetic fields effects on endocytosis and mitosis of cells in culture

M. G. Moisescu[1], P. Levêque[3], L. M. Mir[2], E. Kovacs[1]
[1]"Carol Davila" Medical Univ., Bucharest, Romania, [2]Gustave Roussy Institut - UMR 8121,Villejuif, France, [3]XLIM - UMR 6172, Limoges, France

Cellular nutrition, signaling, transmembrane transport or surface area control are under the influence of complex endocytotic mechanisms. Fluid phase endocytosis is one main type of endocytosis, consisting in engulfment of liquid media into vesicles formed on the cell membrane' inner side. This provides a pathway, for any external soluble molecule, to reach the cell vesicular compartment.
The fluid phase endocytosis of B16 cells was quantified using the fluorescent Lucifer Yellow. The cells were exposed to 900MHz pulsed electromagnetic fields (EMFs) (217Hz envelope frequency, 580μs pulse duration), mean specific absorption rate from 3.2 to 4.2Wkg^{-1}. The exposures were performed into wire patch or Transverse Electric Magnetic (TEM) cells, for times up to 1hr. For cell mitosis studies a TEM cell with different diameters holes into the cell' plates, mounted on an inverted microscope, was used.
Statistical significant increase of 25% of the endocytotic rate under EMFs exposure was observed for times as short as 20 min. Incubations with specific inhibitors of two main molecular pathways of endocytosis (chlorpromazine inhibitor of clathrin-dependent pathway and filipin III inhibitor of caveolin-dependent pathway) suggested that the clathrin-dependent endocytosis is perturbed by the EMFs pulses. No significant perturbations of mitosis duration or different mitosis phases durations were observed. But still increased endocytotic rate was observed for exposures performed into the TEM cell used for mitosis studies.

P-526

Ceramide domains are at the origin of hot-cold haemolysis induced by PlcHR2 from Pseudomonas

L. Montes[1], J. Sot[1], L. A. Bagatolli[2], M. Stonehouse[3], M. Vasil[3], F. M. Goñi[1], A. Alonso[1]
[1]Unidad de Biofísica (CSIC-UPV/EHU) Universidad del País Vasco. Leioa ,Spain, [2]MEMPHYS. University of Southern Denmark, Odense, Denmark., [3]Dpt. of Microbiology. University of Colorado at Denver Health Sciences Center. Aurora,Co USA

Hot-cold haemolysis is the phenomenon whereby red blood cells incubated at 37° C in the presence of certain agents, undergo rapid haemolysis when transferred to 4° C. The mechanism of this phenomenon is not understood. $PlcHR_2$, a phospholipase C/sphingomyelinase, but not the phospholipase C activity, is essential for hot-cold haemolysis because the phenomenon occurs not only in human erythrocytes, that contain both phosphatidylcholine (PC) and sphingomyelin (SM), but also in goat erythrocytes, that lack PC. Fluorescence microscopy observations confirm the formation of ceramide-enriched domains as a result of $PlcHR_2$ activity.and the coalescence of ceramide domains after cooling down to 4° C. We suggest that the formation of these large, rigid domains in the originally flexible cell make it fragile thus highly susceptible to haemolysis. We also interpret the slow haemolysis as a phenomenon of gradual release of aqueous contents induced by SMase activity, These hypotheses are supported by the fact that ceramidase, that is known to facilitate slow haemolysis at 37°C, actually hinders hot-cold haemolysis and by DSC experiments. Finally, in liposomes, that exhibit slow release of aqueous contents at 37° C, addition of 10 mol% ceramide and transfer to 4° C cause a large increase in the rate of solute efflux.

P-527

Transient ordered domains in single-component phospholipid bilayers

T. Murtola[1], T. Róg[1], E. Falck[2], M. Karttunen[3], I. Vattulainen[4]
[1]Helsinki University of Technology, Espoo, Finland, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]University of Western Ontario, London, Canada, [4]Helsinki University of Technology and Tampere University of Technology, Espoo/Tampere, Finland

We report evidence of denser and more ordered transient domains in single-component phospholipid bilayers. The domains were first observed with a coarse-grained model, constructed based on small atomic-scale simulations [J. Chem. Phys. 126, 075101]. We then used larger atomistic molecular dynamics simulations to confirm the presence of the domains and to characterize them in detail [Phys. Rev. Lett. 97, 238102]. The simulations show large fluctuations in the local density of the lipid tails. The length scales range up to the size of the system (20 nm), and the distribution changes on timescales of $\sim$10 ns. Further, the lipid tails within these regions are more ordered and often form smaller highly ordered domains. The sizes of these ordered domains range from a few tails up to a few nanometers, and lifetimes from 10 ps to 10 ns, depending on the size. Our observations shed light on the origin of experimentally observed critical fluctuations close to the main phase transition, and can also be relevant for more complex systems.

P-528

Coexpression of IL-9R with IL-2R and MHC glycoproteins in common superclusters of T lymphoma cells

E. Nizsalóczki[1], G. Vámosi[1], Z. Fazekas[1], N. Szalóki[1], C. K. Goldman[2], T. A. Waldmann[2], S. Damjanovich[1], A. Bodnár[1]
[1]Cell Biology and Signaling Research Group of Hung. Acad. Sci., Dept. of Biophys. and Cell Biology, Univ. Debrecen, Debrecen, Hungary, [2]Metabolism Branch, NIH, Bethesda, USA

IL-9 is a multifunctional cytokine with pleiotropic effects on T cells. Biological effects of IL-9 are mediated by its heterodimeric receptor complexes consisting of the cytokine-specific alpha subunit and the common gamma chain shared with other cytokines including IL-2 and IL-15, important regulators of T cell function. Previously we have shown preassembly of the heterotrimeric IL-2 and IL-15 receptors as well as association of their alpha chains in human T lymphoma cells. Binding of IL-2 or IL-15 altered interactions within the receptor complexes. We have also demonstrated that IL-2 and IL-15 receptors form supramolecular clusters with MHC glycoproteins in lipid rafts of T cells. Here we investigated cell surface organization of IL-9R in Kit225 T lymphoma cells transfected with IL-9Ralpha. By using CLSM and FRET we have shown co-expression and molecular scale association of IL-9Ralpha with IL-2R and MHC molecules in common membrane domains (lipid rafts) suggesting that IL-9Ralpha is another component of the aforesaid superclusters. Effect of cytokine treatment on the interactions of IL-9Ralpha with the shared gamma chains (assembly of IL-9R) as well as with other subunits of IL-2R was also studied. According to our data arrangement of the IL-9/IL-2R subunits can be modeled with a heterotetrameric structure which is modulated upon cytokine binding. This arrangement would allow efficient sharing of the gamma chains by the two receptor kinds.

Abstracts

– Membrane microdomains & signalling –

O-529

Structural modulation of receptor-mediated signalling activity in membrane microdomains

P. O'Shea
Cell Biophysics Group, School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD United Kingdom

This presentation will outline how membrane protein structure may be modulated when resident in membrane microdomains known as rafts, as compared to the fluid mosaic membrane. It seems likely that α-helical geometry is modulated by the presence of a membrane protein within a membrane raft. This phenomenon will be illustrated with examples of how such structural modification then leads to alteration in receptor properties. Thus offering cells the means of modifying receptor function in addition for example, to covalent modifications etc.
An important related question to this hypothesis, therefore, involves the identification of the factors that predispose the assembly and disassembly of membrane rafts. Our group has also addressed this question and some entirely novel mechanisms related to extracellular factors will be outlined. The latter work has yet to be published but indicates that raft assembly is much more than the membrane response to the presence of cholesterol.

P-530

Novel imaging and biological applications of the phase-sensitive membrane dye di-4-ANEPPDHQ

D. M. Owen[1], H. B. Manning[1], S. Kumar[1], D. M. Grant[1], J. McGinty[2], P. M. Lanigan[2], S. Oddos[1], C. Talbot[2], P. Debeule[2], E. Jury[4], M. A. Neil[2], P. M. French[2], A. I. Magee[3]
[1]Chemical Biology Centre, [2]Department of Physics, [3]National Heart and Lung institute, Imperial College London, U.K., [4]Wolfson Institute, University College london, U.K.

Cholesterol enriched microdomains (lipid rafts), are believed to be important for the lateral organisation of the plasma membrane. It is thought that these domains have higher order than the bulk membrane due to the organizing nature of the sterol rings and saturated lipid tails. Several membrane staining dyes exist which report on the lipid phase through changes in their fluorescent properties, the best known of these is LAURDAN which requires two-photon excitation to avoid photo-toxicity. Recently, a new dye has been developed which is also sensitive to the order of its local bilayer environment; di-4-ANEPPDHQ exhibits a 60nm spectral shift between phases and an increase in its fluorescence lifetime in the ordered phase. We have applied di-4-ANEPPDHQ to a variety of systems using a range of novel imaging technologies. These include using hyperspectral fluorescence lifetime imaging (FLIM) to image and characterise di-4-ANEPPDHQ in artificial bilayers and in live epithelial cells. We have also imaged the dynamics of the decrease in order due to cholesterol depletion on a rapid spinning disk confocal FLIM microscope. Other applications include spectral and lifetime measurements at the immunological synapse, membrane nanotubes and primary T cells from patients affected by Systemic Lupus Erythematosus where increased levels of cholesterol in the plasma membrane are suspected.

P-531

Methyl-beta-cyclodextrin does not preferentially target lipid raft cholesterol

I. Parmryd, S. Mahammad
The Wenner-Gren Institute, Stockholm University, Sweden

Methyl-beta-cyclodextrin (MBCD) is frequently used to acutely deplete cells of cholesterol and is a popular tool in lipid raft research, based on the idea that cholesterol is an absolute requirement for lipid raft integrity and that its depletion leads to lipid raft dispersion, i. e. if a cellular process is affected by cholesterol depletion it is assumed that lipid rafts are involved. A widespread assumption is that MBCD preferentially targets cholesterol in lipid rafts. To analyse this systematically, a protocol for progressive cholesterol depletion of Jurkat T cells was established using MBCD and [^{3}H]-cholesterol. Up to 50% cholesterol depletion did not substantially affect the viability of these cells. Depletion of 10, 20, 30, 40 and 50% total cholesterol was performed at 37° and cells were lysed in Triton X-100 and subjected to subcellular fractionation by sucrose density equilibrium centrifugation. MBCD extracted similar proportions of cholesterol from the Triton X-100 resistant (lipid raft enriched) as it did from other cellular fractions. Moreover, cholesterol was not exclusively extracted from the plasma membrane and the cells rapidly reestablished the relative differences in cholesterol concentration between different compartments. Moreover, they restored the cholesterol level in the plasma membrane by mobilising cholesterol from intracellular cholesterol stores. The results clearly show that MBCD does not specifically extract cholesterol from lipids rafts and that intracellular cholesterol stores can be used to replenish plasma membrane cholesterol.

P-532

Spatial distribution of FAK pTyr577 in sensory neurons reveals two types of cell contact to the ECM

M. Paveliev, A. Velthut, M. Saarma
Institute of Biotechnology, University of helsinki, Finland

Extracellular Matrix (ECM) is a crucial regulator of development, plasticity and regeneration in the nervous system. In particular laminin signaling through integrins is required for axonal growth in mammalian sensory neurons. The mechanisms of integrin signaling in neurons are still poorly understood. In the present study we combined confocal microscopy with 3D image reconstruction to reveal spatial distribution of activated focal adhesion kinase (FAK) in dorsal root ganglion (DRG) neurons cultured on laminin substrate. FAK activation was visualized by immunostaining for FAK phosphorylated on Tyr577 (FAK pTyr577). We demonstrate that FAK pTyr577 is enriched in lamellipodia and axonal growth cones. In contrast FAK pTyr577 signal at the cell body – substrate interface is very low through the interface area, but enriched in point-type clusters with characteristic size of less than 300nm. We conclude that two types of neuron – ECM contact: motile lamellipodia and stable cell body – substrate interface have different spatial organization of FAK signaling. Surprisingly FAK pTyr577 is also enriched in clusters below the plasma membrane at those sites where the neuron does not contact the substrate. Nuclei of DRG neurons also exhibit high level of FAK pTyr577 signal suggesting a role for activated FAK in regulation of neuronal gene expression.

Abstracts

– Membrane microdomains & signalling –

P-533

Interaction between endocytosis, drugs size and Pgp transporters in Multi Drug Resistance

C. Rauch[1], A. Pluen[2]
[1]University of Nottingham, U.K., [2]University of Manchester, U.K.

In cells, multi drug resistance (MDR) is associated with Pgp-like transporters expression extruding drugs from cellular membranes. MDR is efficiently generated with a relatively small fraction of membrane transporters. As the insertion of drugs in membrane is widespread, there are no reasons why a drug should incorporate the membrane in the vicinity of a transporter. As a result a further elusive hypothesis is usually invoked: these transporters act like "vacuum cleaners" of drugs embedded in the membrane. Nonetheless, how these transporters attract drugs remains obscure. To clarify the "vacuum cleaner" notion, we suggest that during its residency time in cellular membranes, the lateral movement of drugs from their point of insertion to transporters is governed by Brownian's diffusion, which allows the drugs/transporters interaction. Taking into account the Pgp-like transporters functionality, namely the extrusion of drugs from the plasma membrane inner leaflet, we have characterized how the state of drug resistance is triggered involving: membrane endocytosis, drug physico-chemical properties and Pgp-like transporters surface density. In addition, the theory developed provides for the first time a theoretical proof of Lipinski's second rule with regard to drugs' size (or MW) selectivity on their permeation across cellular membranes.

O-534

Modulation of membrane lateral organization by the interplay of ceramide with lipid rafts

L. Silva, R. F. M. de Almeida, B. Castro, M. Prieto
Centro Química-Física Molecular, IST, Lisbon, Portugal

Following a previous work on the sizes and composition of rafts (defined as the lo phase in the lo/ld phase coexistence region), in sphingomyelin (SM)/ palmitoyphosphatidylcholine (POPC) /cholesterol (chol) membranes [1], the alterations induced by small amounts of ceramide (Cer) was studied using photophysical methodologies [2]. Cer can be generated by the hydrolysis of SM in the membrane in response to several stress signals, and its generation is a nearly universal feature for apoptosis. Thus, the changes induced by Cer in lipid raft properties, are crucial to understand the mechanisms underlying the regulation of cellular processes.
It was observed that: i) when chol content is low, Cer readily induces the formation of highly ordered very small (∼4 nm) Cer/SM gel domains, which became surrounded by rafts (lo phase), upon increasing the Chol/SM content. However the size of the rafts did not change, i.e., Cer can not induce the formation of very large platforms as observed in POPC/Cer binary mixtures, ii) on the other hand, Cer/SM domains are abolished when chol content, and thus l_o fraction, is increased, indicating that the two molecules compete for association with SM. In this last situation, lipid rafts control both the biophysical properties and lateral organization of the membrane.
[1]-de Almeida, RFM, Loura, LMS, Fedorov, A and Prieto, M. (2005) *J. Mol. Biol.* **346**, 1109.
[2]-Silva, L., de Almeida, R. F. M., Castro, B., Fedorov, A., Prieto, M. (2007) *Biophys. J.***92(2),** 502.
[3]-Silva, L., de Almeida, R. F. M., Fedorov, A., Matos, A. P., Prieto, M. (2006) *Mol. Membr. Biol.* 23(2), 137

P-535

Reconstitution of integrin alphaIIbetab3 into giant unilamellar vesicles

P. Streicher[1], M. Bärmann[2], J. Spatz[3], P. Bassereau[1]
[1]Institut Curie, Laboratoire Physico-Chimie Curie, Paris, France, [2]Lehrstuhl für Biophysik E22, Physik Department, Technische Universität München, Germany, [3]Abteilung Biophysikalische Chemie, Universität Heidelberg, Germany

Using a method previously developed in our laboratory, we have reconstituted the transmembrane protein integrin alphaIIbbeta3 into giant unilamellar vesicles (GUVs). In a first step, our method involves a detergent-mediated reconstitution of solubilized membrane proteins into small proteoliposomes. In a second step, these preformed proteoliposomes are partially dried under controlled humidity and GUVs are eventually formed by electroswelling of the partially dried film. The reconstitution process has been validated by analyzing protein incorporation and biological activity. Confocal microscopy showed that the fluorescently-labeled protein were homogeneously incorporated into the membrane. Moreover, after reconstitution, the proteins retained their biological activity as demonstrated by adhesion test onto surfaces covered with RGD, ligand of the integrin alphaIIbbeta3. These very large unilamellar integrin-containing vesicles (more than 20 mm in diameter) will provide unique biomimetic systems for further studies of cell adhesion.
[1] P. Girard et al., Biophys. J. 87: 419-429 (2004)

P-536

A study of the rupture of vesicles with ternary lipid mixtures on SiO2 surfaces by means of QCM-D

M. Sundh[1], B. Kasemo[2], S. Svedhem[2], D. S. Sutherland[1]
[1]iNANO Center, University of Aarhus, Denmark, [2]Department of Applied Physics, Chalmers University of Technology, Sweden

It is well known that ternary lipid mixtures of phosphatidylcholine, sphingomyelin (SM), and cholesterol (chol) phase separate and form domains enriched in SM and chol; so called rafts. Rafts are believed to be involved in numerous cellular processes such as cell signaling, endocytosis etc. and thus the importance of their study. Quartz crystal microbalance with dissipation (QCM-D) is a tool commonly used to study and quantify the adsorption of proteins and the fusion of lipid vesicles into lipid bilayers. In this study the influence of lipid composition in lipid vesicles on the formation of bilayers was investigated and interpreted in terms of the phase segregation of the components. In addition the QCM-D was investigated as a tool to determine lipid phase. Ternary lipid mixtures of POPC/SM/chol at different compositions were formed into single unilamellar vesicles by extrusion and deposited on SiO_2 coated QCM crystals. Preliminary results show that the formation of lipid bilayers can be tuned by changing the lipid composition or temperature. An increase in the concentration of SM results in a reduction in bilayer quality, seen by an increase in the change in dissipation response. These results can be interpreted in terms of phase separation into ordered and disordered fluid domains within the vesicles. As the SM concentration is increased the ordered phase becomes more dominant up to a point where the vesicles are too rigid to fuse and form bilayers. A temperature study shows that rupture of SM rich vesicles could be induced by doing the experiment at increased temperatures and hence changing the lipid phase.

Abstracts

– Membrane microdomains & signalling –

P-537

Polycation-anionic liposome interfacial complexes: physico-chemical characteristics

A. V. Sybachin, A. A. Efimova, E. A. Litmanovich, A. A. Yaroslavov

Moscow State University, Chemistry Department, 119992, Leninskie Gory, Moscow, Russian Federation

Adsorption of a polycation on the surface of mixed liposomes, composed of neutral phosphatidylcholine and anionic cardiolipin, has been examined. The content of cardiolipin (Q) was varied from 5up to 20 mol.%, these molecules being uniformly distributed within each membrane leaflet and between them. The interaction was accompanied by neutralization of the surface charges of liposomes and their aggregation. The amounts of polycation, required for the complete charge neutralization and formation of the largest particles, was linear in Q, thus indicating that all anionic lipid molecules, from both membrane leaflets, were involved in electrostatic binding with cationic units of the adsorbed polymer.

An excess of the polycation, over needed for the complete neutralization, was shown to adsorb on the liposomal membrane. Importantly, the amount of the abundant polymer was independent on the Q value. The PEVP-to-liposome binding was likely terminated when a charge brought in by adsorbed PEVP reached a certain positive value.

Due to lateral segregation and transmembrane migration of lipids, induced by adsorbed polycation, the composition of the interfacial polycation-membrane complexes was the same for all the studied liposomes. A thickness of a layer formed by the adsorbed polycation was found to be approx. 30 mn.

P-538

Re-evaluating the membranes role on the HIV-1 neutralizing monoclonal antibody 2F5 mode of action

A. S. Veiga, M. Castanho

Centro de Química e Bioquímica, FCUL, Ed. C8, 1749-016 Lisboa, Portugal

2F5, a monoclonal antibody that neutralizes HIV-1 primary isolates, recognizes an epitope in the membrane proximal region of the glycoprotein gp41 ectodomain. It is believed that binding to the viral membrane is a step in the antibody mode of action, as usual in ligand-membrane receptor interactions. We investigated the interaction of 2F5 with membrane model systems, namely large unilamellar vesicles, by means of fluorescence techniques. There are no significant interactions of the 2F5 with model viral membranes or with model target cell membranes. Therefore the usual three-step 'membrane catalysis' method is not followed by 2F5 in its mode of action.

P-539

Visualizing association of signaling proteins in lipid microdomains

K. Weise[1], C. Nicolini[1], J. Kuhlmann[2], H. Waldmann[2], R. Winter[1]

[1]Department of Chemistry, Biophysical Chemistry, University of Dortmund, Otto-Hahn-Str. 6, 44227 Dortmund, Germany, [2]Max-Planck Institute of Molecular Physiology, Otto-Hahn-Str. 11, 44227 Dortmund, Germany

Two-photon fluorescence microscopy on giant unilamellar vesicles and tapping-mode atomic force microscopy (AFM) are applied to follow the insertion of a fluorescently labeled and dually lipidated N-Ras proteins with different anchor systems into heterogeneous lipid bilayer systems. Ras proteins are involved in the regulation of cell differentiation, mitosis, growth control, and cell cycle regulation, acting as molecular switches shuttling between active GTP-bound and inactive GDP-bound states. The lipid bilayers consist of canonical raft mixtures, which - depending on the concentration of the constituents - separate into liquid-disordered (l_d), liquid-ordered (l_o) or solid-ordered (s_o) phases. By combining both techniques, we were able to detect partitioning of N-Ras in lipid domains of canonical raft mixtures at length scales from the μm to the nm range. The phase sequence of preferential binding of farnesylated/hexadecylated N-Ras to mixed micro-domain lipid vesicles is $l_d > l_o >> s_o$. The influence of different lipid anchor systems (e.g., hexadecyl/hexadecyl) are discussed. Intriguingly, we detect, using the better spatial resolution of AFM, also a large proportion of the lipidated protein located at the l_d/l_o phase boundary, thus leading to a favourable decrease in line tension that is associated with the rim of the demixed phases (C. Nicolini et al., *JACS* **128** (2006) 192-201). Such an interfacial adsorption effect may serve as an alternative vehicle for association processes of signaling proteins in membranes.

P-540

Striated domains in mixed bilayers of lipids and the model peptide WALP

F. Yarrow, J. P. van der Eerden, T. J. Vlugt

Condensed Matter and Interfaces, Utrecht University, Netherlands

Highly ordered striated domains with a repeat distance of 8 nm are formed spontaneously in mixed bilayers of saturated DPPC-lipids and the transmembrane model peptide WALP in the gel phase, as observed by Atomic Force Microscopy (AFM). Higher and lower lines are visible which consist of alternating rows of peptides and non-tilted lipids, compared to a 30 degree tilt in the non-perturbed bilayer.

The importance of lipid packing effects upon these striated domains has been demonstrated with AFM. Effects of temperature, hydrophobic mismatch between lipid and peptide, lipid acyl-chain unsaturation and lipid tilt have thus been investigated. The ability of the lipids to tilt and the tightness of their hydrocarbon chain packing are crucial for the formation of these highly defined striated domains

The tightness of the lipid packing in the regular DPPC gel-phase creates a situation where the WALP peptide is expelled from this bilayer into strongly defined regions. The lipids bordering these peptides are probably forced the orient themselves parallel to the non-tilted peptides. The width of this perturbed lipid region could be the underlying factor determining the 8 nm repeat distance. Non-tilting lipid systems do not have this tilt-perturbed region and therefore do assemble into localized regions but do not display the highly defined repeat distance.

Currently, information about these mixed lipid/WALP systems is being obtained on a more detailed scale by use of computer simulations on a coarse-grained model of the system.

Abstracts

– Membrane microdomains & signalling –

P-541

Molecular events associated with BLM properties and phylogenetic development of vertebrates.

A. E. Zaqaryan, N. M. Ayvazyan
Dpt. Biophysics, Yerevan State University, Armenia

At 1967 by acad. E. Kreps it was shown that biochemical composition of lipid membranes in nervous tissues of vertebrates is undergone insignificant changes during the hundreds millions years of evolution. Till that time numerous data on the lipid qualitative composition in vertebrate animals indicate that this component has been conserved in the course of phylogenetic adaptation of animals. However, it can be assumed that the processes related to the membrane bilayer (BLM) state underwent certain changes as the structural organization of vertebrates was becoming more complicated.
We isolated lipid fractions from the brain, heart, liver and muscle of vertebrates: crucian carp (*Carassius carassius*), marsh frog (*Rana ridibunda*), caucasian agama (*Stellio caucasicus*), and white rats by the method of Keits. Model membranes were formed from the total lipid fraction on a teflon aperture by the method of Muller. The electrical parameters of the BLMs were determined on an electrometric device equipped with a Keithley 301 differential feedback amplifier (USA) in a voltage-fixation mode. The potential of membrane rupture recorded in the experiments in shielded camera was taken as the threshold value of the voltage applied; 0.1 M KCl, NaCl, LiCl, $CaCl_2$ served as ionic media.
It was discovered, that penetration of bilayers from lipids of nervous tissue decrease in row of poikilotherm vertebrates and increase at mammals. In most cases bilayer's penetration higher for K^+- ions, than for Na^+ and Li^+. The electrical properties of BLMs from heart in media of Ca^{+2} ions demonstrated very high conductivity and we connect this fact with specific function of heart muscle.

Abstracts

– Organelle motility –

O-542

The role of myosin VI in protein sorting and post Golgi membrane trafficking

J. Au[2], M. Chibalina[1], C. Puri[1], S. D. Arden[1], J. Kendrick-Jones[2], F. Buss[1]

[1]Cambridge Institute for Medical Research, University of Cambridge, Hills Road, Cambridge CB2 0XY, U.K., [2]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QR, U.K.

In membrane trafficking pathways vesicles are rapidly moved between different compartments within the cell using a variety of motor proteins moving along networks of actin filaments and microtubules. Myosin VI is a unique motor that moves backwards towards the minus end of actin filaments. This motor plays an essential role in range of intracellular processes such as cell migration, endocytosis, exocytosis as well as cell division. Targeting of myosin VI to specific cellular localisations involves a number of binding partners such as optineurin, Dab2 and GIPC. The C-terminal tail of myosin VI contains two hot spots for cargo binding, a WWY and a RRL motif, each can bind multiple binding partners. In addition, the C-terminal tail domain contains a lipid binding region that binds with high affinity and specificity to phosphatidylinositol-4,5-bisphosphate (PIP2). Functional studies on siRNA treated KD cells have shown that myosin VI is linked to the Golgi complex and the secretory pathway by optineurin, which also binds to Rab8. Over expression of constitutively active Rab8-Q67L recruits myosin VI onto Rab8 positive structures. Since Rab8 has been shown to regulate basolateral transport in polarised epithelial cells we investigated the role of myosin VI in the sorting of membrane proteins in MDCK cells. We observed that the splice isoform of myosin VI with no insert in the tail domain is required for the polarised transport of newly synthesised membrane proteins to the basolateral domain.

P-543

Myosin heads axial orientation in muscle during isotonic and isometric contraction

F. Berenguer[1], A. Svensson[1], J. Bordas[1], J. Juanhuix[1], J. Campmany[1], T. Weiss[2], T. Narayanan[2]

[1]LLS-CELLS. Laboratori de Llum Sincrotro. Universitat Autonoma de Barcelona, E-08193 Bellaterra, Spain, [2]ESRF. European Synchrotron Radiation Facility, F-38043 Grenoble, France

Generation of force and movement by striated muscles involves the interaction of myosin II and actin filaments. During the contractile cycle both myosin and actin filaments undergo structural changes that can be monitored with time-resolved x-ray diffraction patterns of muscle. In particular, the myosin meridional reflections, specially the strongest order on the third myosin layer line (3M) at a rest spacing of 14.34 nm, have been traditionally used as markers for myosin head disposition during various forms of contraction. These reflections consist in cluster of peaks, originated by interference effects between myosin diffraction units disposed at each side of the M-line. Here we use small angle x-ray scattering in whole skeletal muscles in order to explore the different conformations and orientations of the two myosin heads in each pair in different contractile states, extending a previous work done with muscles contracting at the plateau of isometric tension. A theoretical analysis allows the extraction of phase information from the clusters of peaks, from which electronic density maps of the myosin heads can be computed. The results show that during isometric contraction each head of the pair has a distinct and well-defined structural disposition, whilst during isotonic shortening there are several possible orientations. However, both heads in each pair show a similar disposition, different to the orientations found at plateau. These results shed light on the molecular basis of the contractile cycle.

O-544

Actin-based propulsion of GUVs is controlled by Arp2/3 complex and membrane-diffusion of N-WASP

V. Delatour, G. Romet-Lemonne, E. Helfer, M.-F. Carlier

Laboratoire d´Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France

Spatially controlled assembly of actin in branched filaments generates a force responsible for the formation of cell protrusions or the propulsion of intracellular vesicles and pathogens. The propulsive movement of giant unilamellar vesicles (GUVs) functionalized by N-WASP is reconstituted in a biochemically controlled medium, with the goal to understand the nature of the molecular processes that determine motile behavior. We find that actin-based propulsion displays a continuous regime or a periodic saltatory regime, and that the transition between the two regimes is controlled by the concentration of Arp2/3 complex, which branches filaments by interacting with N-WASP at the liposome surface. Using phase contrast and double fluorescence microscopy, we show that saltatory motion is linked to cycles in the distribution of N-WASP at the membrane between a homogeneous and a segregated state. Monitoring the distribution of N-WASP, and comparing it to that of the Arp2/3 complex and actin, we obtain direct information on the molecular mechanism of actin-based propulsion. In particular, we show that actin filaments in the comet tail bind to N-WASP, and that these bonds, mediated by Arp2/3, are transitory. The interaction of N-WASP-Arp2/3 with the filaments drives segregation, and competes with free diffusion. The balance between segregation and diffusion determines whether continuous movement can be sustained. Computed surface distributions, obtained by a theoretical description of this segregation-diffusion mechanism, compare favorably to measured density profiles of N-WASP, Arp2/3 complex, and actin.

P-545

Actin directly accelerates the power stroke of myosin

M. Gyimesi[1], B. Kintses[1], C. R. Bagshaw[2], A. Málnási-Csizmadia[1]

[1]Dept. of Biochemistry, Eötvös University, Pázmány s 1/c, Budapest, H-1117, Hungary, [2]Dept. of Biochemistry, University of Leicester, Leicester, LE1 9HN, U.K.

We found that in myosin II a reversible structural change that can be related to the powerstroke is the rate limiting step of the ATPase cycle and precedes the product release steps. The main consequence of this finding is that actin accelerates the isomerisation step which constitutes the power stroke and not the product release steps directly. Despite M.ADPPi states are weak actin binding forms, the predominant flux of the isomerisation reaction occurs when myosin is attached to actin, because actin activates the isomerisation step and actin binding/release are fast equilibria in these myosin states. Furthermore, we show that the main flux of the release of products is ADP release followed by phosphate release and the flux of the reverse order is negligible therefore the backdoor hypothesis needs reinvestigation. These conclusions are based on transient kinetic and fluorescence characterization of two single tryptophan myosin motor domain constructs, one of which contained a single Trp at the entrance of the ATP pocket (W129+), while in the other one the Trp was located in the relay region (W501+). These constructs allowed us to study ADP and phosphate binding events (W129+) separately from the isomerisation/powerstroke step (W501+).

Abstracts

– Organelle motility –

P-546

Loop 4 of myosin II plays a functional role in weak actin binding states

M. Gyimesi[1], B. Kintses[1], A. K. Tsaturyan[2], M. S. Kellermayer[3], A. Málnási-Csizmadia[1]
[1]Department of Biochemistry, Eötvös University, Institute of Biology, Budapest, H-1117, Hungary, [2]Institute of Mechanics, Lomonosov Moscow State University, Vorobjovy Gory, Moscow 119992, Russia., [3]Department of Biophysics, University of Pécs, Faculty of Medicine, Pécs, H-7624, Hungary

Myosin interacts with actin during its enzymatic cycle and actin stimulates the ATPase turnover of myosin. There are extensive interaction surfaces on both actin and myosin. Several surface loops of myosin have been proven to play different roles in actomyosin interaction. Although loop 4 has been implicated, its functional role remained unknown. We explored the role of loop 4 by either mutating its conserved acidic group, E365, to Gln (E365Q), or by replacing the loop with three glycines (ΔAL) in a *Dictyostelium* myosin II motor domain containing a single Trp residue. We used this native tryptophan (W501) as a sensor of nucleotide binding and lever arm movement. Our results demonstrated that in ΔAL the ATP-induced actomyosin dissociation was highly accelerated and the actin binding K_D increased 2.1-fold in rigor and 3.6-fold in the presence of ADP. V_{max} values of actin-activated ATPase activities were slightly altered but K_M values were increased with 5-fold or 7.3-fold in E365Q and ΔAL, respectively. Furthermore, average *in vitro* actin gliding speeds were accelerated by the mutation and loop deletion. We conclude that loop 4 is a functional actin binding region that plays a role in the stabilization of the actomyosin complex especially in weak actin binding states.

P-547

The effect of phosphate and temperature on isometric force generation of permeabilized fibres of rabbit and dogfish muscle

S.-J. Holohan, T. West, R. Woledge, N. Curtin, M. A. Ferenczi
Imperial College London, NHLI, London, UK

Aim: Previous experiments have suggested that increased intracellular inorganic phosphate (Pi) depresses isometric force production by skeletal muscle. However, most studies of this effect on permeablized fibres have been performed at sub-physiological temperatures. The available data suggest that the inhibitory effects of Pi are reduced as temperature is increased. The aim of this study was to examine the effect of Pi (0-20 mM) on isometric force production at physiological temperatures in white muscle of dogfish and rabbit psoas fibres.
Methods: Experiments were performed using Triton-permeablized single fibres from dogfish white muscle (physiological temperature, 12°C) and rabbit psoas (physiological temperature, 37°C). Fibres were activated from a calcium-rigor state (32 μmol l^{-1} of free Ca^{2+} with backup system) in the presence of 0, 5, 10 and 20 mM Pi in sequence of either increasing or decreasing [Pi].
Results: Peak normalised tension (tension/tension with no added [Pi]) as a function of logarithmic [Pi] showed that (1) for the dogfish there was no statistically significant ($p>0.05$) effect of [Pi] on isometric force output; (2) for the rabbit at 20°C, [Pi] did reduce force significantly ($p<0.005$). However, at 35°C, the effect of [Pi] on force was much smaller than at lower temperatures ($p>0.05$).
Conclusion: At physiological temperatures, the inhibitory effects of phosphate on force generation were reduced compared to those at lower temperatures for single skeletal fibres of dogfish white muscle and rabbit psoas.

O-548

Myosin Structures : What they reveal about the motor mechanism

A. M. Houdusse[1], P. Llinas[1], J. Menetrey[1], H. L. Sweeney[2]
[1]Structural Motility, Institut Curie, CNRS UMR144, Paris, France, [2]Dept. of Physiology, Univ. of Pennsylvania School of Medicine, Philadelphia, U.S.A.

Force is produced by myosin motors upon the actin-driven conformational changes of the motor that leads to sequential release of the hydrolysis products of ATP. Structural information on the multiple states the motor adopt along its motor cycle is essential for understanding how chemical energy is converted into force production. Myosin VI is the most enigmatic of myosins. First, it produces its force and traffics toward the minus-end of actin filament in the opposite direction to other myosins. Second, this motor uses a number of unique mechanisms that are not well understood to take multiple steps on an actin filament without detachment. Surprisingly, these steps are similar in size to those of myosin V, even though the lever arm of myosin VI contains only one IQ motif, whereas that of myosin V contains six. We have recently revealed the structure of the myosin VI motor at the end of its powerstroke (in the rigor conformation). This structure reveals that a specific insert wraps around the converter and binds a calmodulin that interacts with the converter. The result is a $\sim 120^{\circ}$ repositioning of the myosin VI lever arm, which explains its reverse directionality. However, to account for the large powerstroke of myosin VI, this study clearly predicted that the pre-powerstroke state of myosin VI must differ from that of plus-end directed myosins. We have recently solved this structure. It reveals that unexpected rearrangements in the converter are critical to position the lever arm ideally to produce a very large stroke.

P-549

Calcium induced structural rearrangements of spasmin

H. Jankevics, S. R. Martin, J. E. Molloy
MRC National Institute for Medical Research, London, United Kingdom

Some ciliated protists, e.g. *Vorticella convallaria*, have a contractile stalk consisting of an organelle called the spasmoneme. In response to mechanical stimuli this shortens into a folded, helical structure with a maximum speed of 1000 lengths per second (500 times faster than muscle contraction). The contraction process is ATP independent and is driven solely by calcium ions. Changing the calcium concentration in the solution surrounding demembranated spasmonemes results in cycles of contraction and relaxation of the structure. A protein called spasmin has been shown to constitute 40-60% (wet weight) of the spasmoneme. Spasmin shows sequence similarity to centrin, a protein found in microtubule organising centres. It contains two, putative, calcium-binding EF-hands and a 26 amino acid, N-terminal sequence that is predicted to be unstructured. We have characterised the calcium-mediated changes in the structure of spasmin, using circular dichroism (CD) and fluorescence based methods. We found that there is a calcium sensing, high affinity, (C-terminal) site that increases the alpha helicity of spasmin, whilst the largest structural rearrangement of the protein occurs upon calcium binding to the low affinity (N-terminal) site. These structural rearrangements increase the exposed hydrophobic surface, which is thought to be important for inter-molecular interactions. We also found that a truncated form of spasmin (that lacks the N-terminal 26 amino acid extension) binds the 26 amino acid peptide and this increases the calcium affinity of at least one of the sites.

Abstracts

– Organelle motility –

P-550

Reversible movement of switch 1 loop of myosin determines actin interaction

B. Kintses[1], M. Gyimesi[1], D. S. Pearson[2], M. A. Geeves[2], W. Zeng[3], C. R. Bagshaw[3], A. Málnási-Csizmadia[1]

[1]Eotvos University, pazmany peter setany 1/C 1117 Hungary, [2]University of Kent Canterbury, Kent CT2 7NJ, UK, [3]University of Leicester, Lancaster Road, Leicester LE1 9HN, UK.

P-loop NTPases, like G-proteins, myosin, and kinesin, are able to sense the difference between NTP and NDP bound in the nucleotide pocket through the P-loop itself and two additional loops known as switch 1 and 2. Switch 1 loop is believed to mediate the information of the bound nucleotide to the binding site of the partner protein. Structural studies suggested that switch 1 has two conformational states, but their role in the transduction mechanism has yet to be clarified. Using the fluorescence of tryptophan residues introduced into the switch 1 region of myosin II we investigated the behavior of switch 1 during the enzymatic cycle of myosin. We found that in the presence of MgADP, two states of switch 1 exist in dynamic equilibrium. Actin binding, the partner protein of myosin, shifts the equilibrium towards one of the MgADP states, whereas ATP strongly favors the other. In the light of structural results, these findings lead to a model in which the equilibrium constant between the two states of switch 1 is coupled to the strength of the actin–myosin interaction and explain the different effect of ATP and ADP to the actin affinity of myosin. It might have implications for the enzymatic mechanism of P-loop NTPases in general.

O-551

SNARE cluster dynamics influences chromaffin granule motion and probability of fusion

I. López, V. Torres, J. H. Villanueva, S. Viniegra, L. M. Gutiérrez
instituto neurociencias, universidad miguel hernández-cSIC, Alicante, Spain

Expression of SNAP-25 fused to GFP was instrumental to prove the implication of the C-terminus of this SNARE in exocitosis. GFP-SNAP-25 wild type and Δ9 form, product of BoNTA action, were employed to understand SNARE complex implication in vesicle mobility and fusion in bovine chromaffin cells. GFP-SNAP-25 constructs under TIRFM, shows membrane patches of 500 nm average diameter. These clusters contain also syntaxin-1 and synaptobrevin II. Interestingly, the Δ9 form presented similar size and density, but increased lateral and z mobility. Thus, SNARE patches formed with this truncated form tend to move at longer distances from the membrane and in an increased range compared with wild type clusters. This increase in SNARE dynamics correlates with the enhancement of dynamism found for associated chromaffin granules stained with the acidic dye lysotracker red. In addition, single vesicle fusions granules was much improbable in the highly mobile vesicles present in the cells expressing GFP-SNAP-25 Δ9 and with slower fusion kinetics. Therefore, our study suggest that the expression of this truncated SNARE affects, the mobility of SNARE clusters, the motion of the vesicles, and the probability of exocytosis of such granule population.

This work was supported by grants from the Ministry of Science and Technology (MST, BMC2002-00845 and Ministry of Education and Culture (MEC) of Spain/Fondos FEDER(BFU2005-02154/BFI), and the Generalitat Valenciana (GRUPOS 03/040 and ACOMP06/036).

P-552

Branched and unbranched actin filaments: mechanical characterisation and effect on membrane deformation

M. Safouane[1], L. Blanchoin[2], C. Sykes[1]

[1]Laboratoire Physico-Chimie "Curie", UMR 168, Section de Recherche, Institut Curie, Paris, France, [2]Institut de Recherches en Technologie et Sciences pour le Vivant –Laboratoire de Physiologie Cellulaire Végétale (PCV), UMR 5168, CEA Grenoble, France

Actin filament polymerisation generates forces capable of deforming the cell and powering its movement. In protrusive structures, multiple actin filaments are arranged in dendritic arrays (lamillipodia) or parallel bundles (fillipodia). The goal of our study is to characterize the force generation by actin either in cross-linked networks, or in bundled structures. We will present a mechanical characterization of the actin filaments using a magnetic tweezer. Depending on their reticulation, and on the presence of different proteins involved in actin polymerization (Arp2/3 complex, formins), different rigidities in the kPa range are observed. Moreover, we will present preliminary experiments on actin polymerization at a membrane surface.

P-553

Theoretical modeling of cortical actin instabilities

G. Salbreux[1], P. Pullarkat[2], J. Prost[1], J.-F. Joanny[1]

[1]Physicochimie Curie (CNRS-UMR168), Institut Curie, France, [2]Physikalisches Institut, University of Bayreuth, D95440-Bayreuth, Germany

The actin cortex is made of a thin crosslinked actin gel underlying the cell membrane. Actin is treadmilling in the cortex and polymerizes from the cell membrane. Actin also interacts with myosin molecular motors which generate internal stresses in the actin layer. The actin cortex thus controls the cell shape and allows for mechanical deformations of the cell. We use the "polar active gel model", which gives a mesoscopic description of actomyosin systems, to account for some experimentally observed cortical instabilities. We discuss the regulation of the cortex thickness in a spherical geometry, the mechanism of bleb formation and an example of fibroblast oscillation in which the cortical layer interacts with mechanosensitive calcium channels.

Abstracts

– Organelle motility –

O-554

The Glideosome: the machinery powering gliding motility and host cell invasion in apicomplexan parasites

D. Soldati[1], F. Plattner[1], W. Daher[1], M.-F. Carlier[2]
[1]Dept. of Microbiology and Molecular Medicine, CMU, Univ. of Geneva, Geneva, Switzerland., [2]Lab. d´Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France

The Apicomplexans are obligate intracellular parasites and include important pathogens such as *Plasmodium* and *Toxoplasma gondii*, responsible for malaria and toxoplasmosis. Apicomplexans rely on gliding motility for their migration across biological barriers and for host cell invasion and egress. This unusual substrate-dependent mode of locomotion involves the concerted action of a myosin motor, factors regulating actin dynamics, secretory adhesins and proteases. During invasion, complexes of soluble and transmembrane micronemes proteins (MICs) are discharged to the apical pole of the parasite, they bind to host cell receptors and redistribute towards the posterior pole of the parasite via a physical connection to the parasite actomyosin system.
The susceptibility of the Apicomplexa to actin-polymerizing and actin-depolymerizing drugs implies the existence of a machinery that promotes actin nucleation and polymerization. Apicomplexans lack the ARP2/3 complex but possess formins and a profilin that can possibly form a processive motor capable of assembling F-actin. *T. gondii* profilin has been identified as a modulator of the innate immune response via the activation of the Toll-like receptor-11 but is also fulfilling an essential role in parasite motility and invasion. The inducible knockout of this gene offers a unique opportunity to dissect the dual function that this protein is playing during the establishment of infection. We have compared the functional and biochemical properties of the profilin from different Apicomplexa and are currently investigating the functional contribution of the formins in the process of invasion.

P-555

Particles moving along actin stress fibers visualized by scanning probe microscopy

K. Tamura, T. Mizutani, H. Haga, K. Kawabata
Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan

Intracellular directional transportation of molecules and organella is essential process for cell activities such as cell migration, absorption and secretion in epithelial cells, and nervous system formation. The polar cytoskeletal filaments composed of actin filaments and microtubules allow various kinds of cargos including transport vesicles and organella to move directionally. In the case of actin filament-based transportation, cargos move in the short range of submicrons, while in the case of microtubule-based transportation those do in the long range of several microns. Although actin filaments play an important role in directional transportation, the role of actin filaments is not fully understood.
We successfully acquired the time-lapse images of actin filament network in migrating fibroblasts by the contact-mode scanning probe microscopy (SPM). We found that many particles of several hundreds nanometer diameter appeared and moved rather in the long range of several microns along actin stress fibers. In the process of cell migration, the actin filament network was rearranged actively in the lamella region and organized into actin stress fibers. The number of particles in the SPM images decreased when the actin stress fibers were established. Actin filaments were concentrated in the particles by the fluorescence staining of the actin filaments. Therefore, we consider that the transportation of the moving particles is a new type of directional transportation related with actin stress fibers.

P-556

Vertical cell motility monitored by noise analysis of thickness shear mode resonators and atomic force microscopy

M. Tarantola, A. Janshoff
Institute of Physical Chemistry, Ak Prof. Dr. A. Janshoff, Jakob-Welder Weg 11, Johannes-Gutenberg-University Mainz, 55128 Mainz, Germany

Invasive and metastatic behaviour of malignant cells is the major cause of mortality in all cancer patients and therefore detection of cell motility important for understanding the metastatic process and developing clinical measurements for treatment of cancer. As a fundamental property of mammalian cells is their motility, it is possible to distinguish benign cancer cells from malign ones by their reduced motility and also different cell lines from each other; a quantification of the metastatic potential is achieved by the usage of acoustic shear wave resonators, which permit an analysis of frequency fluctuations correlated to the vertical motility of the cells, i.e. the dynamics of forming and breaking focal contacts without net lateral movement.
We showed how the Quartz-Crystal-Microbalance (QCM) could be used to monitor these shape fluctuations of living cells: we found that mammalian MDCK-II cells grown on TSM resonators show characteristic fluctuations on the resonance frequency. Applying noise analysis to the fluctuating resonance frequency yields power density spectra and the dynamic activities of the cells impose several characteristic resonances; monitoring of dissipation factor in a self-made QCM-D setup and network-analysis allows comparison of time courses of dissipative processes. Impedance analysis again was employed to control confluence of the cell monolayer. Influence of synchronisation in the cell cycle as well as the initial cell densities (inoculum) on the signal was scrutinized.

P-557

Conformational changes of dynein-arms in situ studied using x-ray diffraction of flagellar axonemes

S. Toba[1], H. Iwamoto[2], T. Fujisawa[3], H. Sakakibara[1], K. Oiwa[1]
[1]Kobe Adv. ICT Res. Cntr., Japan, [2]JASRI, SPring-8, Japan, [3]RIKEN Harima, Japan

We have carried out X-ray diffraction studies on whole flagella isolated from *Chlamydomonas* and have obtained structural information of the axoneme. We used several types of flagella prepared each from strains of wild type, and mutants lacking the whole outer arm (*oda1*), spoke (*pf14*), and central apparatus (*pf18*). We aligned axonemes in a flow cell by continuous shear flow, and then obtained diffraction patterns with the synchrotron radiation x-ray of 0.9Å wavelength at SPring-8, BL45XU. The diffraction pattern showed several distinct meridional and equatorial reflections. Compared under the same nucleotide conditions, the 24-nm meridional reflection of the wild type was stronger than that of *oda1*, suggesting that this reflection originates mainly from the axial repeat of the outer dynein arms. The weaker 32-nm meridional reflection of *pf14* compared with the wild type suggests that this reflection mainly comes from the repeat of the spoke. The 19-nm equatorial and 16-nm meridional reflections of *pf18* were weaker than in the wild type, and these changes were presumably caused by the lack of the central sheath in *pf18*. Diffraction patterns were also obtained in different nucleotide states: no nucleotide, AMP-PNP, and ADP/V_i. In the ADP/V_i state, the 24-nm meridional reflection became over twice as strong as that in the no nucleotide state. We calculated diffraction patterns from the electron density map of an outer-arm-microtubule complex obtained by cryo-electron tomography. The patterns reproduced well the equatorial, 24-nm and 12-nm meridional reflections. Comparison of these diffractions indicate some nucleotide-dependent axial/azimuthal motions of outer dynein arms.

Abstracts

– Organelle motility –

P-558

Is zero magnetic field influencing human spermatozoa dynein molecular motor ?

Z. Truta[1], S. Lerintiu[2], M. Garlovanu[2], R. Micu[2], V. V. Morariu[3]
[1]Faculty of Physics, Babes-Bolyai University, 400084, Cluj-Napoca, Romania, [2]I.V.F. Laboratory, Cluj-Napoca, Romania, [3]INCDTIM, 4000293, Cluj-Napoca, Romania

Human haploid male germ cells (spermatozoa) have their own means of motility using a dynein type molecular motor. Analyzing UV-VIS spectrum from a Earth geomagnetic field (GMF), and a zero magnetic field (ZMF) exposed human semen we noticed as a qualitative information that glucose is consumed from spermatozoa faster in ZMF. Basic quantitative analysis showed that glycolysis is accelerated in ZMF, and as a result, the glycolysis related production of ATP. How the kinematic parameters of spermatozoa and semen ATP level are correlated, a basic theory for the influence of ZMF on kinematic parameters is suggested. Our previous work suggested the same [1].To verify this path of kinematic parameters possible influence, we conducted a set of experiments on 2 groups of patients known with oligozoospermia , and normozoospermia, using a computer asisted manual trajectory reconstruction method. Our statistical results, the first to our knowledge, prove that basic ZMF influence on human spermatozoa kinematic parameters is mainly due to the increase glycolysis related production of ATP. Path velocity (VAP) and curvilinear velocity (VCL) values increase as a result to ZMF exposure.

References:
1.**Z.Truta**,Silvia Neamtu, V.V.Morariu, Zero magnetic field influence on in vitro human spermatozoa cells behavior, Rom.J.Biophys.,15, (1-4), 73-77,(2005)

P-559

the biophysical mechanics of SNARE-mediated membrane fusion.

C. E. Turner[1], O. Ces[1], P. S. Freemont[2], R. H. Templer[1]
[1]Chemistry Department, Imperial College London, U.K., [2]Division of Molecular Bioscience, Imperial College London, U.K.

Neurotransmitters are released from the synapse of neurons when vesicles containing them fuse with the plasma membrane. The proteins thought to be vital in this step are the SNARE proteins; Syntaxin, SNAP25 and VAMP. Here we present the establishment of an in-house in vitro model based on these proteins. Current work has reproduced published data suggesting the importance of membrane properties on the rate of fusion. It has been shown that increasing curvature elastic stress, through introduction of lipids with negative spontaneous curvature, increases the rate till a plateau is reached. Increasing the bending rigidity of the membrane also causes a rise in the fusion rate but upon further addition the rate falls quickly.

Abstracts

– Interaction and recognition of DNA –

P-560

Force-induced structural transitions in cross-linked DNA films

A. André, T. Fischer, G. Maret, T. Gisler
University of Konstanz, Konstanz, Germany

Single-molecule experiments have revealed that double-stranded DNA can be extended by about 60% beyond its natural contour length at nearly constant force. [1,2]. The origin of this plateau in the force-extension curve is however still under debate, mainly since no direct structural information has so far been obtained from single overstretched DNA molecules.
We propose an experimental approach to investigating the structure of overstretched DNA, using oriented DNA films obtained by wet-spinning. Randomly cross-linking DNA by intercalation compounds and covalent bonds between nucleobases results in elastomeric films which can be reversibly overstretched. We characterize the structure of these cross-linked films under mechanical load with X-ray diffraction and birefringence experiments.
[1] P. Cluzel, et al., "DNA: An Extensible Molecule," Science **271**, 792-794 (1996).
[2] S. B. Smith, et al., "Stretching DNA beyond its B-form contour length," Biophys. J. **68**, A250 (1995).

P-561

Ab Initio Analysis of Vibrational Spectra of Nucleic Acid Complex with Cisplatin

V. Andrushchenko, P. Bour
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic

Cis-Diamminedichloroplatinum(II) (cisplatin) is a widely used anticancer drug [1]. DNA is considered as its main target in the cell. Such a drug binding significantly distorts the DNA double-helix and inhibits the replication. A number of techniques have been used to elucidate effect of cisplatin on DNA. Recently, a relatively novel vibrational circular dichroism spectroscopy (VCD) has been used to study cisplatin complexes with a DNA octamer d(CCTGGTCC)•d(GGACCAGG) [2]. Despite the general ability of VCD to provide enhanced information about molecular geometry, the analysis of the experimental spectra is intricate. The interpretation becomes more straightforward and unambiguous by comparison with appropriate theoretical simulations. In the present work we employed combined MD/QM calculations using vibrational properties of large molecular fragments. Infrared (IR) and VCD spectra of a DNA octamer d(CCTGGTCC)•d(GGACCAGG) with and without cisplatin were simulated. A comparison to experimental data contributed to understanding of DNA modifications induced by the cisplatin complexation. Spectral changes resulting from a distortion of the DNA double-helix could be modeled separately from those caused by the drug attachment only. Most of the observed DNA octamer vibrational bands could be assigned.
[1] Cohen, S.; Lippard, S.J. Prog. Nucl. Ac. Res. and Molec. Biol. 2001, 67, 93-130.
[2]Tsankov, D.; Kalisch, B.; van de Sande, H.J.; Wieser, H. Phys. Chem. B 2003, 107, 6479-6485.

P-562

Bistable regime of ligands binding with macromolecules induced by external noise

A. V. Arakelyan[1], V. B. Arakelyan[2]
[1]Biology Departament, Yerevan State University, Yerevan, 0049 Armenia, [2]Yerevan Physics Institute, Yerevan, 0036 Armenia.

It is considered that during binding of ligands with a macromolecule, the structure of adsorption center on a macromolecule is changing continuously. This brings to the necessity of adsoprtion description with two non-linear differential equations. The first equation of balaced type describes binding of ligands with a macromolecule, and the second one describes relaxation of depth of adsorption hole on a macromolecule. We consider that ligand - DNA complex formation and dissosiation is fast process, therefore we can reduce the number of equations, keeping only relaxation equation, which contains parameters of potential hole and ligand - macromolecule binding parameters. In contrast to numerous publications, we examine more realistic case, where parameters of medium are fluctuating. We approximated external noise as Gaussian white noise. Then we obtained expression for stochastic potential, which depends on external noise. When the intensity of external noise is small, stochastic potential has one minimum, but when the external noise has high intensity stochastic potential has two minimums. This fact leads to bistable regime of the ligands binding with macromolecule, which is induced by external noise.

P-563

Electrostatic models of the wrapping of DNA around oppositely charged proteins

L. Arcesi, G. La Penna, A. Perico
Consiglio Nazionale delle Ricerche (CNR), Institute for Macromolecular Studies (ISMAC), Via De Marini 6, 16149 Genova, Italy

We propose electrostatic models for interactions DNA-oppositely charged proteins. DNA is modeled as a negatively charged cylinder and the protein as a sphere with an uniform positive surface charge. The interaction is mediated by the condensation/release mechanism of the counterions provided by salt solution. The binding free energy of this DNA-sphere model is given as the difference of the system free energy with a length l of DNA wrapped on the sphere, to that without any wrapping and is a sum of different terms. The former contribution is the free energy in the condensation regime of the piece of straight DNA, the second the electrostatic energy of a spherical macroion, after bending around it a length l of DNA, plus the electrostatic interaction between straight DNA and the complex sphere–wrapped DNA, and the latter term is the elastic energy necessary to bend a piece of DNA of length l, corrected by the additional electrostatic repulsion due to DNA bending. The length of wrapped DNA around the sphere at the equilibrium is obtained by searching the minimum of the binding free energy. These models well explain the wrapping of DNA around the histone octamer in the nucleosome and the bending of linker DNA in chromatin, due to interactions with the N-terminal tail of histone H3. We also present an electrostatic motivation for the CSB protein active remodelling of chromatin by wrapping and unwrapping of DNA depending on ATP binding. Finally we show how electrostatics compact DNA in archaea by binding to the histone-like protein MC1.

Abstracts

– Interaction and recognition of DNA –

P-564

Effects of different dose zinc diet on taste sensation

K. Aydin[1], I. Ozcan[1], D. Ozcelik[2], M. C. Akyolcu[2]

[1] Istanbul University Faculty of Dentistry Department of oral diagnosis and radiology, Istanbul, Turkey , [2]Istanbul University Cerrahpasa Medical Faculty Department of Biophysics, Istanbul, Turkey

Nutrition is one of the vital functions of survival for all species. The most important parameter of the nutrition of the human and animals is the taste sensation. Between different societies and animal species different responses against sweet, bitter, salty and sour tastes is related to the nutrition habits and palate taste. In tasting the most important factor is distribution and the function of the taste papilla on tongue. In nutrition pyramid, vitamin, mineral and ion utilization take very important place besides proteins, carbohydrates and lipids. Several studies stressed the important role of the zinc in nutrition and tasting. Present study was planned to determine the possible role of the zinc deficiency in nutrition preference of the experimental rats.

The daily utilized food, which contains different concentration of zin, amount by rats was determined. Four groups of Sprange-Dawley type rats, weighing 170-220 gm, were undergone to the experimental process. By calculating the daily water utilization of the animal, modified LSD test was applied for statistical analysis.

There are statistically significant differences between group I and groups III, IV and between group II and groups III and IV. from the daily water utilization point of view. As a result of present study it may be concluded that zinc carries out important role in sufficient nutrition

O-565

Condensation of single DNA molecules by multivalent ions observed using magnetic tweezers

K. Besteman, S. G. Lemay

Kavli Institute of Nanoscience, Delft, The Netherlands

The condensation of stiff, highly-charged DNA molecules into compact structures by condensing agents ranging from multivalent ions to small cationic proteins is of major biological and therapeutic importance, yet the underlying microscopic mechanism remains poorly understood. We probed DNA condensation by simple multivalent ions and protamines using a combination of bulk and single-molecule experiments. Using magnetic tweezers, we studied in real time the condensation of single DNA molecules under tension. We found that DNA condensation occurs via discrete nucleated events; by measuring the influence of an imposed twist, we showed that condensation is initiated by the formation of a plectonemic supercoil. This demonstrates a strong interplay between the condensation transition and externally imposed mechanical constraints. Using electrophoretic mobility measurements, we further showed that the effective charge of condensed DNA reverses sign at high concentrations of condensing agents. Our magnetic tweezers measurements indicate that this charge inversion behavior influences condensation by modulating the barrier for condensate nucleation. These observations are consistent with the theoretical proposal that charge inversion and condensation are linked, purely electrostatic phenomena driven by the existence of a strongly correlated liquid of counterions at the DNA surface.

P-566

Direct visualisation of DNA aggregates by confocal and polarising microscopy: sequence effects

N. J. Brooks[1], R. E. Robson[1], A. Goldar[1], G. S. Baldwin[1], S. Leikin[2], J. M. Seddon[1]

[1]Imperial College London, London, UK, [2]National Institutes of Health, Bethesda, USA

While many important studies involving DNA have been carried out under dilute aqueous conditions, *in vivo* DNA is found in a highly compact condensed state. Of particular interest are condensed structures formed by DNA fragments with specific sequences. These may help elucidate the mechanism by which DNA can recognise a homologous sequence: a highly important step in many biological processes including genetic repair and gene shuffling.

Two DNA sequences have been produced by PCR amplification from ϕX174 DNA. These are each 294 base pairs long and have similar GC content. Each sequence has been independently condensed in aqueous solution using polyethylene glycol (PEG); polarising microscopy shows that these form small cholesteric spherulites. 1:1 mixtures of the two different sequences can also be condensed with PEG to form cholesteric spherulites, but in this case, significant disruption of the cholesteric structure is observed.

Samples of each DNA sequence have been labelled with two fluorescent dyes, mixtures of these have been condensed using PEG. Labelled DNA samples show the same behaviour as their unlabelled analogues by polarising microscopy. Labelled samples were examined by confocal fluorescence microscopy. Mixtures containing each fluorescent dye attached to the same DNA sequence show complete mixing of the two dyes. However, mixtures containing two different sequences each labelled with a unique dye display significant spatial separation between the fluorescence from each dye.

P-567

The influence of cis-DDP on the formation of the complexes between DNA and some nuclear proteins

E. V. Chikhirzhina[1], E. I. Kostyleva,[1], V. I. Vorobyev[1], A. M. Polyanichko[2]

[1]Institute of Cytology, RAS, Russian Federation, [2]Saint-Petersburg State University, Russian Federation

Today cisplatin (dichlorodiamminoplatinum(II)) is one of the most successful anti tumor drugs. This platinum coordination compound is able to form several types of stable adducts with DNA. Cisplatin binding changes the structure of DNA and prohibits its proper functioning in living cell. Some nuclear proteins (e.g. HMGB proteins) can recognize cis-DDP-DNA adducts. Non-histone chromosomal proteins HMGB1/2 are the members of a large family of High Mobility Group proteins and provide additional levels of structural and functional complexity. Despite the fact that HMGB1/2 present in the cells of all investigated organisms their functions remain unclear. We have studied the conformational changes of DNA upon the interaction with non-histone chromatin proteins HMGB1 and HMGB2 in presence of cis-DDP using UV Circular Dichroism (CD) and UV absorption spectroscopy. We have shown that despite the similarity in their primary structures the mechanisms of the interactions of HMGB1 and HMGB2 with DNA are different, that might determine the differences in their functioning in the living cell. The properties of DNA CD in the complexes with HMGB1 are determined by the interaction of DNA with cis-DDP. CD of DNA-HMGB2 complexes mostly depends on DNA-protein interactions. HMGB2 may interact not only with adduct sites on DNA but also with platinum-free regions; and/or HMGB2 has higher affinity to DNA damaged by cis-DDP, compared to the affinity of HMGB1, that would lead to the considerable distortion in DNA structure.

Abstracts

– Interaction and recognition of DNA –

P-568

The conformations of the ribosomal protein S15 and the protein interaction with 16S RNA

T. Créty[1], T. E. Malliavin[2]

[1]Institut de Biologie Physico-Chimique, Paris, France, [2]Institut Pasteur, Paris, France

The interaction between the ribosomal protein S15 and its binding sites in the 16S RNA, was examined from two points of view. First, the isolated protein S15 was studied by comparing NMR conformer sets, available in the PDB and recalculated using the CNS-ARIA protocol. The recalculation of the S15 NMR structure, as well as the recording of MD trajectories from a conformer of each set, reveals that several orientations of the N terminal helix α1 with respect to the structure core are populated. Trajectories of the complex between the ribosomal protein S15 and RNA were also recorded in presence and in absence of ions Mg^{2+}. The ions Mg^{2+} are penta- or hexa-coordinated by water and RNA oxygens and mainly interact with the RNA phosphodiester backbone, reducing the RNA mobility and inducing electrostatic screening. When the ions Mg^{2+} are removed, the internal mobility of the RNA and of the protein increases at the interaction interface close to the RNA G-U/G-C motif, due to a gap between the phosphate groups in the UUCG capping tetraloop and to the disruption of S15-RNA hydrogen bonds in that region. On the other hand, several S15-RNA hydrogen bonds are reinforced and the network of hydrogen bonds observed in the loop between α1 and α2 is consequently reorganized. In absence of Mg^{2+}, this network has the same pattern as the network observed in the isolated protein, where the helix α1 is mobile with respect to the protein core. The presence of Magnesium ions may thus play a role in stabilizing the orientation of the helix α1 of S15.

O-569

Zones composed of short adenine and thymine words correspond to SIDD sites in bacterial sequences

G. Damiani

Faculty of Pharmacy of the University of Genoa, Italy

We describe the application of a string based computer program developed by us to the search in *E. coli* and other bacterial sequences regulatory sites composed of short strings of A and T (AT words, ATW). Such sites correspond to the SIDD sites.

The program performs the following operations: 1) Conversion of the DNA sequence to a linear vector of A, G, C and T characters; 2) Scan of the vector from the first to the Nth element with a five Char window and construction of a string containing the five Chars read at each scan; 3) Formation of a matrix with permutation of the A and T Chars in 5 positions; 4) At the end of scan, saving in the output file the matrix with the occurrences of each ATW. ATW length is measured as the sum of overlapped 5 base words, *e. g.* word AAATTTT is composed of AAATT, AATTT, ATTTT, and its ATW length is 3. Total length of ATW present within consecutive 100 bps patterns was plotted and the peaks of ATW compared to the peaks present in the SIDD profiles obtained by submitting the same sequences to the Web SIDD facility. A correspondence of the AT peaks to the SIDD sites was always present. In one of the tested sequences, ECILVGME, the ATW peak site strictly corresponds to the regulatory sequences present in Regulon DB. Moreover, the strong topological correlation of the groups of short ATWs found with the SIDD sites points to the recognition of functional sites by our method.

P-570

Molecular mechanisms of the biological synergism of DNA-intercalating drugs

M. P. Evstigneev[1], A. O. Rozvadovska[1], D. B. Davies[2]

[1]Department of Physics, Sevastopol National Technical University, Ukraine, [2]School of Biological and Chemical Sciences, Birkbeck College, University of London, U.K.

A number of aromatic DNA-targeting drug molecules exert a pronounced biological synergism when used in combination. Well-known examples are simultaneous administration of Caffeine, Chlorophylline or Vitamin B_2 with an aromatic antibiotic or mutagen. Interpretation of the observed biological effects at molecular level has been made in terms of the 'interceptor action' as a result of direct interaction between the drug and Caffeine/Chlorophylline (i.e. hetero-association) [Piosik J. et al, Biochem. Pharm. 63 (2002) P.635]. Such interpretations commonly result from separate studies of two-component Drug-(polymeric DNA) and Drug-Interceptor complexation in solution.

In the present work we have made a series of ^{1}H NMR spectroscopic investigations of both two-component (Drug-DNA, Drug-Drug) and three-component (Drug-Drug-DNA) complexations of a range of aromatic drug molecules (including antibiotics, mutagens and vitamins) with an oligomeric DNA sequence used as a model of non-histone- region cellular DNA. The results show that, in addition to the interceptor action, the competition of the drugs for DNA binding sites (protector mechanism) needs to be taken into consideration [Evstigneev M.P. et al, Biophys. Chem. 118 (2005) p.118] and that, for some combinations of drugs, the protector mechanism dominates over the action as an interceptor [Evstigneev M.P. et al, Eur. Biophys. J. 36 (2006) p.1].

P-571

The study of the mechanisms of interaction of the thrombin with DNA aptamers

T. Hianik[1], S. Ponikova[1], I. Grman[1], M. Antalik[2]

[1]FMFI UK, Mlynaska dolina, Bratislava, [2]Institute of Experimental Physics, SAV, Watsonova 47, Kosice

In this work we studied the properties of DNA aptamers with high affinity to α-thrombin. The novel methods based on thickness shear oscillations were applied to study how the ionic strength, type of cation, presence of heparin and the structure of the aptamer affect the affinity of thrombin to the aptamer. We showed, that binding of thrombin to the aptamer immobilized on a surface of the quartz crystal is accompanied by decrease of series resonant frequency and by increase of the motional resistance. This suggests that the binding event is accompanied not only by increase of the mass of the sensing layer, but also in an increase of shearing viscosity. We showed, that ionic strength, pH and presence of heparin substantially influence the binding affinity. We used also the circular dichroism (CD) to study the formation of G-quadruplexes in two types thrombin binding aptamers. We showed, that aptamers forms compact tetraplex structure with antiparallel strand orientation in the presence of potassium cations. This was readily identified through characteristic CD spectra of anitiparallel quadruplex conformation. We also investigated CD spectral changes at various temperatures. At low temperature conditions without potassium we observed similar CD spectra as with stabilizing potassium cations. The intensity of CD bands at low temperature were lower than in the presence of potassium. This suggests that aptamers form quadruplex structure but it is not well-ordered as in presence of cations.

Abstracts

– Interaction and recognition of DNA –

P-572

A methodology to isolate single mitochondria to determine the source of heteroplasmy of mitochondrial DNA

R. B. Kishore[1], J. E. Reiner[2], T. Albanetti[3], K. H. Deckman[3], B. C. Levin[4], K. Helmerson[1]
[1]NIST, Physics Laboratory, Gaithersburg, MD 20899, U.S.A., [2]NIST, EEEL, Gaithersburg, MD 20899, U.S.A., [3]Gettysburg College, Chemistry Department, Gettysburg, PA 17325, U.S.A., [4]NIST, CSTL, Gaithersburg, MD 20899, U.S.A.

Mitochondrial DNA heteroplasmies have been linked to numerous chronic symptoms of mitochondria-based diseases; however, the mechanism producing heteroplasmy is not understood. While heteroplasmies have been studied at the multi-cellular level, the question of whether the heteroplasmy is present within single mitochondria remains to be answered. A single mitochondria contains multiple copies of its genome, therefore it is possible that the heteroplasmy exists even at the single mitochondria level. To address this issue we developed a protocol to isolate single mitochondria from single human leukocyte cells. HL-60 cells, which were shown to contain a heteroplasmy at the cellular level (PCR and sequencing showed a 50/50 C/T heteroplasmy at nucleotide position 12071), were labeled with Mitotracker Green FM. A pulsed UV laser was used to lyse an individual cell, allowing the mitochondria to escape. Optical tweezers was then used to transfer a single mitochondria, which was identified by fluorescence microscopy, into a micropipette tip. PCR and sequencing was subsequently performed on the single mitochondria. This procedure was repeated for a random sample of single mitochondria from an individual cell. Our preliminary results suggest that the heteroplasmy also occurs at the single mitochondria level. Our technique opens up the possibility of determining the origin of mitochondrial DNA heteroplasmy.

P-573

Formation of hairpin structures for DNA hexamers

V. V. Kosjukov[1], D. V. Ovchinnikov[2], D. B. Davies[3], M. P. Evstigneev[1]
[1]Department of Physics, Sevastopol National Technical University, Ukraine, [2]Dep. of Organic Chemistry, St.Petersburg Technological Institute, Russia, [3]School of Biological and Chemical Sciences, Birkbeck College, London, U.K.

The shortest DNA, which can form a hairpin structure in solution, has been found previously to be the heptamer d(GCXYZGC), where the nucleotide triplet X-Y-Z constitutes the hairpin loop and the base pairing of two flanking GC pairs forms the hairpin stem. It has also been demonstrated that the shortest known loop is formed by a dinucleotide sequence X-Y with a triplet stem of d(CGC/GCG). We have shown that formation of a hairpin structure is also possible for the hexamer d(GCATGC) having a $d(GC)_2$ stem and a dinucleotide loop d(AT) but that it is unlikely with the inverted stem $d(CG)_2$. In the present work we have demonstrated by ^{1}H NMR spectroscopy and MD simulations that formation of a hairpin also is possible for d(GCTAGC), which has the inverted loop d(TA). Further MD simulation of hairpins within the family d(GCXYGC) in an aqueous environment leads to the following conclusions for formation of hairpin structures for a DNA hexamer:
- there are no principal sterical constraints on the folding into a hairpin structure for linear nucleotides, d(GCXYGC); inversion of the flanking nucleotides makes the structure unfavourable;
- at least two factors appear to be responsible for the stability of the hexamer hairpin in solution: enthalpy (the vertical stacking of the bases) and an entropic factor (the flexibility of the linear monomer)
- the most stable hairpins for d(GCXYGC) are those with X,Y = (A,C or T).

O-574

Structure-dependent interactions between DNA double helices

S. Leikin
National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892, USA

Interactions between highly charged DNA double helices are involved in some of the most fundamental biological processes such as packing of meters of genetic material within cells and phage heads and recognition of homologous genes in recombination and DNA repair. The chiral packing of DNA in aggregates clearly shows that direct DNA-DNA interactions substantially depend on the molecular structure up to several nanometers of surface separation. The helical structure of the molecules is the only possible source of such chirality. Based on idealized models of helices, we are now beginning to understand how such structural dependence of intermolecular interactions can be described and what role it may play in a variety of other observed phenomena. But, DNA is not an ideal helix. Its structure depends on the base pair sequence. Do intermolecular interactions depend on the sequence as well? Do they in turn affect the molecular structure of the double helix as predicted by recent models? Do such direct, structure-dependent interactions between DNA play any role in cellular functions? I will review the most recent developments in the theory, simulations and experiments, providing new insights into these problems.

P-575

Preparation of dense arrays of end-tethered DNA on solid substrates

H. Li, J. Koota, I. Seuffert, A. André, G. Maret, T. Gisler
Universität Konstanz, Dept. of Physics, 78457 Konstanz, Germany

We discuss various routes to produce dense end-tethered arrays of long-chain DNA molecules to solid substrates, using DNA carrying end groups such as biotin or thiol which specifically bind to surface-anchored streptavidin or gold.
Conventional end-tethering by adsorption of coiled DNA results in low tethering densities and mushroom-like conformations [1] due to the entropic repulsion of the coils. Using DC electric fields applied via conducting substrates such as gold or streptavidin-coated indium-tin oxide, DNA can be driven to the surface by electrophoresis. However, the high viscosity of the high DNA concentration near the surface dramatically slows down the tethering, resulting in only moderate enhancements of the tethering density.
As an alternative approach we exploit the liquid crystalline order in DNA solutions induced by osmotic stress [2] or by convective deposition onto a pinned contact line occurring in a "coffee ring" [3]. We investigate the effects of end-functionalization and end-tethering on liquid crystalline textures, and resulting tethering densities.
[1] R. Lehner, J. Koota, G. Maret, and T. Gisler, Phys. Rev. Lett. **96** (2006), 107801.
[2] R. Podgornik, H. H. Strey, K. Gawrisch, et al. Proc. Natl. Acad. Sci. USA **93** (1996), 4261-4266.
[3] I.I. Smalyukh, O.V. Zribi, J.C. Butler, et al., Phys. Rev. Lett. **96** (2006), 177801.

Abstracts

– Interaction and recognition of DNA –

P-576

Single-molecule biosensors for transcription-factor detection

K. Lymperopoulos, M. Heilemann, A. N. Kapanidis
Clarendon Laboratory, Dept. of Physics and IRC in Bionanotechnology, Oxford University, Parks Road, Oxford, OX1 3PU, U.K.

Transcription factors are proteins that bind to specific regions on DNA and regulate gene expression. Detection of transcription factors can provide essential information about gene regulation, address fundamental biological problems such as development and cell-commitment, and lead to ultrasensitive diagnostics.
Here, we describe a single-molecule fluorescence assay that detects transcription factors with high sensitivity and specificity. The single-molecule assay is based on protein-detected coincidence of two DNA fragments (Heyduk & Heyduk, *Nat Biotech* 2002, 20:171), each containing one half-site for transcription-factor binding; this coincidence is easily detected using alternating laser excitation spectroscopy (Kapanidis *et al*, PNAS 2004, 101:8936). In the absence of a transcription factor, the two DNA fragments (which carry short and complementary single-stranded DNA tails) diffuse independently in solution. In the presence of a transcription factor specific to the fully assembled DNA site, the two DNA fragments diffuse as a single molecular complex; such species can be easily differentiated from the free DNA half-sites and can be counted, reporting on the presence and concentration of transcription factors. Using this assay, we demonstrated protein-dependent DNA coincidence to detect transcription factors in dilute (sub-nM) solutions; multiplexing capability by detecting recognize two transcription factors simultaneously in the *same* solution; compatibility with complex biological samples such as nuclear extracts; and sensing of changes in gene expression in bacterial cells.

O-577

Integron Integrases:Broad DNA specificity and bacterial adaptation

D. MacDonald[1], G. Demarre[2], M. Bouvier[2], D. Mazel[2], D. N. Gopaul[1]
[1]CNRS URA 2185, Institut Pasteur, 75724 Paris, France, [2]CNRS URA 2171, Institut Pasteur, 75724 Paris, France

Integron integrases mediate the integration of foreign DNA on mobile gene cassettes by recombining attC sites with an attI site on the chromosome. Integron integrases are part of the tyrosine recombinases family. We have determined by SAD methods, the crystal structure of the *Vibrio cholerae* integron integrase VchIntIa in complex with a substrate based on the *V. cholerae* cassette repeats (VCR) folded bottom strand to 2.8 Å resolution. This structure reveals the complexity of broad DNA specificity of the integrase. The imperfect palindrome in the substrate results in extruded bases that are stabilized by conserved residues. Thus the G20" and T12" bases are bound in stacking interaction by W157, W219 for the former, and H240, H241 and P232 for the latter. Biochemical and genetic characterization of the binding reaction, and site directed mutagenesis followed by in vivo recombination frequency measurements, have confirmed the essential role of several key residues on both DNA substrate and integrase. The crystal structure highlights that the recognition of folded single stranded DNA by the integrase is part the paradigm for the enzyme to recognize several DNA sequences through the tertiary structure adopted by these DNA molecules. This strategy may facilitate the recognition and integration of multiple gene cassettes, with implication in the adaptation and evolution of bacteria. *Nature* 440 (2006) 1157-1162.

P-578

Investigation of ionic strength influence on the binding of the Actinomycin D derivative to DNA

Y. O. Minakova, O. B. Kruglova
Institute of Radiophysics and Electonics of the N.A.S. of Ukraine 12 Acad. Proskura str., Kharkiv, 61085, Ukraine; e-mail: evgenia_minakova@mail.ru

Actinomycin D is highly potent antitumor agent which has been used as a chemotherapy drug for treating certain types of cancer. Earlier it was shown [1] that the ActII* (a derivative of Actinomycin D) binding to native DNA forms two types of complexes. They assumed these are externally bound and intercalated ligand at low and high values of DNA-ligand concentration ratios correspondingly. Absorption spectra in visible range of this ligand bound to native and denatured DNA do not differ much qualitative. This fact evidences that the second type of complex is not intercalation. It can be external monomeric binding complex. Using computer optimization program of spectophotometric concentration dependences we calculated spectral and thermodynamic (constants and site sizes of binding) parameters in the ActII*–native DNA and ActII*–denatured DNA systems taking into account ability of cooperative binding of this ligand. We show that the adjacent ActII* ligands change absorption spectrum of monomeric bound ActII* ligand. We estimated influence of Na+ ions and other monovalent positive charge ions (counterions) on the drug interaction with DNA using competitive binding model of two ligands with polynucleotide matrix. We also developed a new procedure and appropriate computer programs which allow to calculate the binding parameters in the system ligand-DNA at their multimodal binding.
1 – V. Maleev, M. Semenov, E. Kruglova, T. Bolbukh, A. Gasan, E. Bereznyak, A. Shestopalova J. Mol. Struct 645, 145 -158 (2003)

O-579

Structural organization of the DNA-HMGB1 complexes revealed by VCD and AFM studies

A. M. Polyanichko[1], E. V. Chikhirzhina[2], Z. Leonenko[3], M. Amrein[3], V. I. Vorobyev[2], H. Wieser[4]
[1]Dep. of Physics, St. Petersburg State University, Russian Federation, [2]Institute of Cytology, RAS, St.Petersburg, Russian Federation, [3]Dep. of Cell Biology & Anatomy, University of Calgary, Canada, [4]Dep. of Chemistry, University of Calgary, Canada

High Mobility Group protein HMGB1 is a member of an abundant family of DNA-binding architectural chromosomal proteins. HMGB DNA-binding motif was identified in many chromatin proteins in which it was responsible for structural distortions in DNA. Earlier we have shown that the C-terminal acidic 'tail' of HMGB1 not only modulates the DNA-binding activity of the protein, but also affects the formation of higher order protein–DNA structures. However, the most commonly used structure-sensitive techniques, as UV circular dichroism (ECD) for instance, are not useful for such complexes due to the high level of light scattering of their solutions. To overcome that, we used a combination of IR absorption and vibrational CD (VCD).Spectroscopic and AFM data revealed the construction of highly ordered supramolecular complexes between DNA and a 'tail-less' HMGB1 protein at higher ionic strength. These complexes consist of condensed highly ordered DNA-protein particles. In both spectroscopic and AFM imaging experiments this structural order was observed in a narrow protein to DNA ratio interval. The mechanisms of formation of the observed structures might be similar to the DNA-protein structures previously described for HMGB-related proteins. We also discuss the interaction of HMGB1 with DNA in presence of histone H1.

Abstracts

– Interaction and recognition of DNA –

P-580

Structural studies of KorA and its interactions with KorB and DNA

K. V. Rajasekar[1], A. L. Lovering[2], L. E. Bingle[1], F. Dancea[4], D. J. Scott[3], S. A. White[1], C. M. Thomas[1], E. I. Hyde[1]

[1]School of Biosciences, University of Birmingham, U.K., [2]University of Birtish Columbia, Canada, [3]University of Nottingham, U.K., [4]Institute for Cancer Studies, School of Biosciences, University of Birmingham, U.K.

The IncP (Incompatibility group P) plasmids are low-copy number plasmids that can transfer to and be stably maintained in almost all gram negative bacteria. They carry several antibiotic resistance genes and so are important carriers in the spread of antibiotic resistance. Gene expression in the IncP-1 plasmids is controlled by a network of four global repressors KorA, KorB, KorC and TrbA. KorA and KorB interact co-operatively at several promoters, resulting in more stringent control than can be achieved by a single protein. The aim of this work is to determine the structure of the KorA repressor and the molecular basis of its interactions with DNA and KorB. The KorA protein is a homodimer with two domains – a small dimerisation domain and a DNA-binding domain containing a helix-turn-helix motif. ^{15}N relaxation studies and X- ray crystallography show that the two domains are separated by a flexible linker. Using SAXS with the X-ray structures, we have built a model of the free protein in solution. In the presence of DNA, the DNA-binding heads of the dimer move to straddle the operator but the linker still retains some flexibility. We have shown that KorA interacts directly with KorB *in vitro*, in the absence of DNA. The region of KorA that interacts with KorB lies on the opposite side of the KorA dimer to the operator DNA.

P-581

Application of linear dichroism to DNA kinetics

M. Rittman, M. R. Hicks, A. Rodger
University of Warwick, UK

Linear dichroism (LD) is a type of UV absorbance spectroscopy. It is the differential absorbance between two perpendicularly oriented polarisations of light, and requires an oriented sample. We use micro-volume Couette flow cells to orient the sample.

LD can give information about DNA conformation, secondary structure of proteins and orientation of structural motifs. It is complementary to a number of other analytical techniques in biochemistry, and is particularly useful for fibrous and membrane proteins which are difficult for conventional techniques. It can also be used to measure kinetics of DNA reactions, such as enzymatic restriction and action of topoisomerases.

Here we present experimental data and modelling based on the kinetics of a number of DNA systems studied by LD. For example, when a restriction enzyme cuts a super-coiled DNA plasmid it becomes linear and orientation greatly increases, increasing the LD signal. We have measured this in real time and produced a model for the kinetics which has been solved to find the rate constants. Other systems investigated include PNA binding to DNA, FtsZ polymerisation and persistence length of DNA.

O-582

Spontaneous separation of homologous DNA duplexes in a protein-free environment

R. E. Robson[1], N. J. Brooks[1], A. Goldar[1], A. Wynveen[1], G. S. Baldwin[1], J. M. Seddon[1], S. Leikin[2], A. A. Kornyshev[1]

[1]Imperial College London, London, UK, [2]National Institutes of Health, Bethesda, USA

DNA naturally exists within condensed phases as a mechanism for compaction and organisation of genetic material. We have been studying the condensation of DNA of specific DNA sequences into spherulites under mild osmotic stress, for (i) mixtures of two different DNA sequences, and (ii) monohomologous DNA. In the mixed samples, each DNA sequence is labelled by a unique fluorescent dye, green or red. Monohomologous samples were tagged with both green and red dyes. Colour separation was observed in mixed samples, but not in homologous ones, unambiguously demonstrating that DNAs of the same sequence group together. A rationalization of these findings can be based on the electrostatic theory of interaction of helical molecules in solution. This theory has established the possibility of attraction of two DNA molecules from a distance through an *electrostatic zipper* mechanism and explains a possibility for electrostatic recognition of homologous genes [1].

The observation of DNA separation without proteins is highly significant for DNA recognition. Such recognition is vital in homologous recombination, an essential DNA repair event that is also responsible for gene shuffling, a key process determining evolution and genetic diversity. A persistent mystery has been how DNA molecules are able to find each other prior to recombination. In this report we experimentally demonstrate that DNA can find other DNAs of the same sequence without the aid of proteins.

1. A.A.Kornyshev, S.Leikin, *Phys.Rev.Lett.,*86, 3666 (2001).

P-583

The role of electrostatics in the B to A transition of DNA: from solution to assembly

L. Rudd, D. J. Lee, A. A. Kornyshev
Imperial College London, U.K.

DNA may undergo a transition from its usual B-form to a structurally distinct conformation, the A-form. Two important differences between the conformations are that the A-form possesses a much narrower narrow groove and a reduced separation of phosphates on each strand. B to A transitions tend to be observed in dense aggregates and in alcohol-water mixtures. We shall discuss a simple, physical model that allows us to describe the B to A transition of DNA in both the mixed solvent and dense aggregate environments. As well as electrostatic interactions between phosphates and counterions we include counterion entropy and a conformational energy term which describes the change in sugar conformation in the DNA backbone. In the absence of an environment the B-form is favoured. In the mixed-solvent solution the transition is driven by the interaction of phosphates and counterions across the narrow groove. Once a critical fraction of ions has been localized near the narrow groove, electrostatics favours the A form. At a critical value of the dielectric constant such interactions become sufficiently powerful to induce the transition. In dry aggregates we include a new element, helix-specific inter-molecular electrostatic forces. Inter-helical interactions are sufficient to warrant the transition to the *A* form, unless enough counterions are localized in the narrow groove (which stabilizes the B form). So, in dry aggregates inter- and intra-molecular interactions conspire, in most cases, to keep DNA in the *A* form. The analysis gives a broad picture of the B to A transition, explains some experimental features that are otherwise mysterious and sets a number of new research goals.

Abstracts

– Interaction and recognition of DNA –

O-584

The nucleosome: A transparent, slippery, sticky and yet stable DNA-protein complex

H. Schiessel
Instituut Lorentz, Universiteit Leiden, Leiden, The Netherlands

Roughly three quarter of eucaryotic DNA are tightly wrapped onto protein cylinders organized in so-called nucleosomes. Despite this fact a large amount of the wrapped DNA needs to be accessible since DNA is at the heart of many crucial life processes. I focus here on physical mechanisms that might allow nucleosomes to perform a great deal of such processes, specifically (1) on unwrapping fluctuations that give DNA-binding proteins access to the wrapped DNA portions without disrupting the nucleosome as a whole, (2) on corkscrew sliding along DNA and some implications and on (3) tail-bridging induced attraction between nucleosomes as a means of controlling higher-order folding. Among various points to be discussed I will mention how the underlying basepair sequence of the DNA can be recognized by the nucleosomes on purely mechanical grounds.

P-585

Sensitive detection of oligonucleotides and their adsorption on amalgam and titanium surface

V. Vetterl[1], J. Vaněk[2], S. Hasoň[1], R. Silvennoinen[3]
[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic, [2]Stomatological Research Center, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [3]Department of Physics and Mathematics, University of Joensuu, Joensuu, Finland

The application of a gold amalgam-alloy electrode (AuAE) for a sensitive voltammetric detection of different oligodeoxynucleotides (ODNs) containing the purine units within the ODN-chains in the presence of copper ions is described. Titanium is frequently used as a biomaterial for hard tissue replacement, such as dental and orthopaedic implants. The surface morphology influence the final interactions of the implant with the surrounding environment. Recently, we have investigated the application of diffractive optical element (DOE), which is a computer-generated hologram fabricated using advanced electron beam lithography techniques, for sensing a diversity of material properties. From the EIS, voltammetric and optical measurements the conclusions about the adsorption of the purine (AAG)12 and pyrimidine (TTC)12 oligonucleotide on titanium was delivered. This project was supported by the grant project No.1M0528 of the Ministry of Education and Sport of the Czech Republic an by the grant project KAN200040651 of the Grant Agency of the Academy of Sciences of the Czech Republic.

Abstracts

– Bionanomaterials & design –

P-586

Wormlike micelles "biogels" for the sustained release of drugs

H. Afifi, C. A. Dreiss
Pharmaceutical Science Research Division, King´s College London,150 Stamford Street, London SE1 9NH, UK

The amphiphilic nature of surfactants drives their aggregation into a range of self-assembly structures. Under specific conditions, these micelles can grow into elongated 'polymer-like' flexible aggregates, known as wormlike micelles. Their entanglement into a transient network above a critical concentration imparts them remarkable viscoelastic properties, which have attracted considerable interest, both for technological applications and fundamental studies, due to their analogy with polymer solutions. Their use in pharmaceutical formulations however is still inexistent. In this project, we propose to use biocompatible and biodegradable surfactants forming wormlike micelles, in association with hydrophobically modified polysaccharides, to develop novel hydrogels for topical drug delivery.
Polyoxyethylene cholesteryl ether surfactant was used in combination with a range of non-ionic surfactants with a small head-group, which promoted micellar growth. Rheological measurements of the micellar solutions revealed a Maxwellian behaviour, typical of wormlike micelles, while at specific compositions gel-like properties were observed. Structural investigations were carried out with light scattering and small-angle neutron scattering, which confirmed the growth of the micelles into elongated aggregates. Subsequently, the ability of the micelles to solubilize two pharmaceutical oils, ethyl butyrate and ethyl caprylate, was investigated. Significantly different phase behaviour was observed, the oil with the longest alkyl chain driving micellar growth at lower co-surfactant concentrations.

P-587

Networks of plasma membrane vesicles – a direct approach to study cell constituents on the nanoscale

B. Bauer, O. Orwar
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

Giant unilamellar lipid vesicles in general and specifically nanotube-vesicle networks (NVN) have proven to be exceptionally useful to study chemistry in confined biomimetic compartments. Nanotubes are suspected to play a significant role in intracellular processes, and are also found to interconnect cells, possibly facilitating intercellular transport. In order to achieve properties similar to the plasma membrane, incorporating membrane proteins is essential. This is usually done by reconstitution of membrane proteins in lipid vesicles, which has several drawbacks. Most importantly, the environment around these proteins is artificially created, as the vesicles are derived from lipid mixtures. We demonstrate that NVNs can be constructed directly from plasma membrane vesicles of single cells where vesicle diameters and tube lengths are in the micrometer range. This approach sidesteps common reconstitution problems as the lipid environment and membrane protein content is preserved, but still controllable by molecular biology techniques. For example, by expressing a light driven proton pump a proton gradient can be established in the derived vesicle, providing the driving force to generate ATP in the NVN. To fully exploit the possibilities we use a novel membrane proteome profiling tool in combination with LC-MS to characterize the protein composition of the plasma membrane as well as of the derived vesicles of the respective cell line. This system provides a direct means to study transport activity across membranes (protein-mediated) and nanotubes (diffusion), as well as substrate conversion down to the single-molecule limit.

P-588

Bio-inspired silica formation via membrane templating

A. Bernecker[1], R. Wieneke[2], A. Geyer[2], M. Sumper[3], C. Steinem[1]
[1]Institut für Organische und Biomolekulare Chemie, Georg-August-Universität Göttingen, Germany, [2]Fachbereich Chemie, Philipps-Universität Marburg, Germany, [3]Lehrstuhl für Biochemie I, Universität Regensburg, Germany

Silica biogenesis proceeds at mild physiological conditions with greater control of pore size, shape and micropatterning than it is possible with typical industrial sol-gel methods. Outstanding examples of hierarchically organised nanostructures are the silica exoskeletons of diatoms, unicellular, eukaryotic algae. Their cell walls are formed within hours from naturally-occurring precursors and are composed of amorphous, hydrated silica associated with organic compounds. Among these, diatom cell wall proteins, so-called Silaffins, have been identified to be involved in silica biogenesis. Templates for the biomimetic synthesis of ordered silica structures are obtained by using the self-assembly properties of lipids in membranes by which defined domain structures are formed. We use a Silaffin-analogue compound, namely membrane-anchored L-Lysinol, which has silica-precipitating abilities. A synthetic glycolipid which does not precipitate silica is used as matrix molecule in membranes. Fluorescent microscopic investigations of monolayers at the air-water interface showed domains in the micrometer-range due to phase separation of the two lipids. The monolayers were immobilised by Langmuir-Blodgett-technique and studied by fluorescence and scanning force microscopy. Silica formation after addition of monosilicic acid induced by Lysinol-containing membrane systems was detected by QCM-D-technique and spectroscopic ellipsometry. The obtained structures are examined by scanning electron and scanning force microscopy.

O-589

Functional interfaces between biomolecules and nanomaterials

T. Cass
Imperial College, London, U.K.

The current excitement associated with nanoscale science and technology derives from the novel properties of materials with characteristic length scales in the range from 1-100nm. Biomolecules also lie within this size range and so there is much interest in building hybrid nanostructures that combine nanomaterials and biomolecules.
Given the diversity of both nanomaterials structures and biomolecules building functional interfaces between the two will never be a 'turnkey' process and yet there are general principles of both surface chemistry and biomolecular structure that should allow a rational 'design' approach to be taken. In the case of nucleic acids (at least as single stranded probes) the absence of a defining 3-D structure, the availability of synthetic derivatives and their polar nature makes the attachment to nanostructured surfaces relatively straightforward. Proteins offer much more of a challenge not only in terms of their often marginal stability of the folded (functional) state compared to the unfolded state, particularly when the latter can bind non-specifically (through van der Waals interactions) to the surface but also because of the diversity of their surface properties.
In this lecture I will illustrate how nanomaterials can be modified with biomolecules in ways that retain both the structure and function of the biomolecule and the novel properties of the material. Examples of nanomaterials will include metals, metal oxides and polymers and the general approach will be to build complementary surface chemistries into the two components such that the binding between them is dominated by a single molecular interaction.

Abstracts

– Bionanomaterials & design –

P-590

Iron influence on structure and biocompatibility of aluminosilicate bioglasses

S. Cavalu[1], V. Simon[2], F. Banica[1]
[1]University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania, [2]Babes-Bolyai University, Faculty of Physics, Cluj-Napoca, Romania

Ferrimagnetic glass-ceramics are successfully applied to reinforce the bone and to decrease the recurrence of tumors by hyperthermia treatment. The ferrimagnetic nanoparticles developed in the vitroceramic biomaterial cause heating through hysteresis losses or magnetic relaxation phenomena. Because the process depends on ferrimagnetic crystals size, a rigorous structural characterization of these vitroceramics must be done. Sol-gel method was chosen to prepare the aluminosilicate materials containing iron in different concentration. This method allows obtaining non-crystalline materials at much lower temperatures than that used in the classical melting methods. These structural disordered precursors are suitable for controlled crystallization and development of magnetic iron oxide phases. This study is focused on FTIR, FT- Raman spectroscopy and thermal analysis of Al_2O_3-SiO_2-Fe_2O_3 system, followed by simulated body fluid investigation as bioactivity evidence. The biocompatibility was evaluated with respect to proteins adsorption, by FTIR measurements and deconvolution techniques. The type and amounts of adsorbed proteins (fibrinogen, albumin) mediate subsequent adhesion, proliferation and differentiation of cells as well as depositing of mineral phase. The deconvolution procedure reveals the conformational changes of the adsorbed proteins and the results are compared and discussed taking into account that lower [sheet]/[turn] ratio indicates inferior blood compatibility.

P-591

Glial scar mechanics – SFM elasticity measurements of "physical barriers"

A. F. Christ, K. Franze, J. R. Guck
Department of Physics, University of Cambridge, U.K.

Glial scars form almost inevitably at sites of injury to the central nervous system (CNS). In contrast to injuries to the peripheral nervous system, the functional recovery of CNS neurons after trauma is highly improbable. Inhibition of neurite regrowth is ascribed to the glial scar. On the one hand, glial cells in the damaged region secrete a multitude of chemical signals – some of which may be potent inhibitors to neurite extension. On the other hand, glial scars are often called a "physical barrier". However, the term "physical barrier" is not clearly defined and is often used differently depending on the context.

There exists an increasing awareness for the sensitivity of biological cells to the mechanical properties of their environment. For example, neurite extension and branching are increased on softer substrates compared to stiffer ones, and neurites and synapses in the retina seem to be confined to those tissue layers where glial cells are softest. We will quantify the elasticity of glial scar tissue and cells using scanning force microscopy (SFM) and compare the measurements to healthy tissue. This will enable us to test whether the "physical barrier" is indeed a "mechanical barrier" and to rebuild mechanically plausible glial scar models *in vitro*. Finally, our experiments might result in the suggestion of new medical strategies treating CNS trauma, including the "physical" rather than focusing solely on the "chemical" barrier aspect.

O-592

SU-8 patterned chips for controlled film formation: lipid monolayer spreading and DNA immobilization

Y. Erkan[1], I. Czolkos[1], A. Jesorka[1], P. Dommersnes[2], M. Wilhelmsson[1], O. Orwar[1]
[1]Department of Chemical and Biological Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden, [2]MSC, Université Paris Diderot, 10, rue Alice Domon et Léonie Duquet, F-75205 Paris, France

Many applications in biotechnology and bioanalysis are based on surface interactions, and controlling surface properties is therefore of great importance. We introduce two novel and related techniques for formation of molecularly thin films based on (i) spreading and mixing of lipid monolayers from multilamellar vesicles and (ii) a one-step procedure for high-yield immobilization of cholesteryl-tetraethyleneglycol conjugated ssDNA (chol-DNA). The films were produced on planar chips with micrometer-sized hydrophobic SU-8 patterns surrounded by a hydrophilic gold layer. Both, immobilization of chol-DNA and formation of lipid monolayer films exclusively take place on SU-8. A single multilamellar vesicle can be placed site-specifically on an SU-8 structure where the lipid spreads as a monolayer due to the high surface tension between SU-8 and the aqueous environment. Next, we show that chol-DNA adsorbs from solution to the SU-8 structures due to hydrophobic interaction, leaving the surrounding gold surface free of chol-DNA. Chol-DNA immobilization is complete within 15 min and yields a surface coverage in the range of 20–95 pmol/cm^2. Furthermore, immobilization is stable after dry-storage for several hours and complementary DNA hybridizes efficiently to immobilized chol-DNA. Our findings offer a new approach to dynamic surface functionalization and decoration as well as surface based-catalysis and self assembly.

P-593

Entrapment of membrane-bound ion channels in sol-gel glasses: a fluorescence study

R. Esquembre, J. A. Poveda, R. Mallavia, C. R. Mateo
I.B.M.C. Universidad Miguel Hernández de Elche, Alicante, Spain.

Proteins entrapped in sol-gel matrices have been extensively studied during the last 15 years showing that the majority of them can be encapsulated with retention of their native structure and functionality. The resulting bioceramics are permeable to a wide variety of molecules and are highly suitable for optical and electrochemical applications. To date, the sol-gel technology has focused mainly on soluble proteins but not on membrane proteins. The major problems limiting the immobilization of the latter systems are due to their lower stability, as well as the necessity to develop a methodology able to retain the physical properties of the lipid bilayer, since it is the media where membrane proteins perform their activity. Recently, we have demonstrated that use of alcohol-free sol-gel routes combined with negatively charged lipids could minimize effects exerted by host matrix on liposome structure, increasing its stability (Esquembre et al. J. Phys. Chem. B. 2007). Here we use the same experimental protocol to immobilize the transmembrane ion channel polypeptide gramicidin in a sol-gel matrix. Gramicidin was reconstituted in anionic liposomes and its immobilization was confirmed from changes observed in the emission spectra and fluorescence lifetimes of their tryptophan residues. Ion channel activity was determined using the fluorescent dye pyrene-1,3,6,8-tetrasulphonic acid and long term stability of the immobilized system was checked by measuring the steady-state fluorescence anisotropy of tryptophan and that of the extrinsic membrane probe diphenylhexatriene. This study constitutes the first stage for future works with more clinically relevant membrane proteins.

Abstracts

– Bionanomaterials & design –

P-594

Density profile and reorientational dynamics of proteins adsorbed at a polyelectrolyte brush

O. Hollmann, C. Czeslik
Physikalische Chemie I, Universität Dortmund, Germany

Polyelectrolyte brushes, consisting of long poly(acrylic acid) (PAA) chains that are grafted to a solid support, exhibit a unique protein binding affinity: At low ionic strength, proteins adsorb spontaneously at a PAA brush, whereas the addition of a few 100 mM salt to the protein solution leads to drastically reduced binding affinity. Most strikingly, this effect of salt appears to be universal for all proteins regardless of the sign of their net charge. Here, we present neutron reflectivity experiments that report on the density profile of alpha-lactalbumin that is adsorbed at a PAA brush. The adsorbed protein can be localized inside the whole PAA brush, although it has a net negative charge and is interacting with the brush under electrostatic repulsion condition. Increasing the temperature reduces the amount of adsorbed protein indicating an exothermic adsorption process. In addition, total internal reflection fluorescence anisotropy measurements of different proteins at a PAA brush are reported. It has been found that the anisotropy of the positively charged lysozyme is increasing when the temperature is raised, whereas that of the negatively charged proteins BSA and alpha-lactalbumin is decreasing. Furthermore, the reorientational correlation time of lysozyme decreases upon adsorption, whereas that of BSA and a-lactalbumin is slightly increasing. It is suggested that positively charged proteins interact with the PAA chains via a complexation mechanism. In contrast, negatively charged proteins are immobilized at a PAA brush by a partial release of their positive counterions thereby maintaining a high degree of reorientational freedom.

P-595

Zwitterionic oil-in-water (o/w) microemulsions for use as drug delivery vehicles

C. M. Hsieh[1], C. Dreiss[1], M. J. Lawrence[1], R. Heenan[2]
[1]Pharmaceutical Sciences Division, King´s College London, London SE1 9NH, [2]Rutherford Appleton Laboratory, Chilton, Didcot OX11 0QX, U.K.

Microemulsions(μe) are thermodynamically stable dispersions of immiscible phases(oil and water) stabilized by amphiphile(s). Due to their transparency, ease of preparation and stability, μe are of interest as drug delivery vehicles. In this study we have investigated the use of o/w μe as vehicles for poorly-water soluble drugs. O/w μe were prepared using the pharmaceutical oils, ethyl butyrate(EB) and ethyl caprylate(EC), and the non-toxic, zwitterionic surfactant 3-(*N,N*-dimethyldodecylammonio)propanesulfonate(DDAPS). The molecular architecture of the μe was determined using a combination of light scattering(LS) and small angle neutron scattering (SANS) studies in combination with contrast variation. At DDAPS concentrations of 5 wt% or less, LS showed the micelles and μe to be 'spherical' droplets. At higher DDAPS concentrations, larger, rod-like structures were present. SANS studies showed that the aggregates were 'short' rods, even at low DDAPS concentrations. Both the radius and the length of the rods were seen to increase with increasing oil and surfactant. Despite the 'apparent' differences in aggregate shape with the two scattering techniques, the size of the aggregates determined by SANS and LS were in agreement. When comparing μe containing equal volume fractions of oil, the EC μe were larger than those containing EB, which were of similar size to the DDAPS micelles, implying the EC μe may be most suitable for drug delivery. The results of this study will aid the optimization of μe for drug delivery.

P-596

Polymerisable lipids for patterned tethered bilayer membranes

M. Jung, W. Knoll, I. Köper
Max Planck Institute for Polymer Research, Mainz, Germany

Various model membranes have been developed in the past to study the physical and physiological properties of biological membranes. Tethered bilayer membranes combine an inorganic support, accessible by various surface analytical tools, a hydrophilic spacer, acting as ionic reservoir below the bilayer and hydrophobic chains derived from natural phospholipids, building the starting blocks of the bilayer

We have developed a modular system, which is suitable for gold or oxide surfaces. These systems serve as a quasi-natural environment for the study of membrane proteins. Functionality of the membrane proteins could be shown by electrochemical methods.

We now aim to control the membrane formation according to a chip-like patterned substrate. This step would allow the creation of protein arrays over different sensing areas. The basic idea is to selectively polymerise the membrane leaving small fluid areas where the proteins can incorporate.

We synthesised lipopolymers, bearing polymerisable sorbyl or diacetylene groups in the hydrophobic part of the lipid, offering at the same time the possibility to be anchored to the substrate. The cross-linking of these new molecules in the plane of the substrate is light induced with a ultra-violet lamp. The possibility to illuminate through a mask makes this procedure very versatile. We could follow the polymerisation process with electrochemical impedance spectroscopy observing the increase in membrane resistance. This shows the enhancement of the membrane stability and indicates a reduced fluidity in the polymerised parts.

P-597

Hemolytic activity of polyamidoamine dendrimers

B. Klajnert, S. Pikala, M. Bryszewska
University of Lodz, Dpt. of General Biophysics, Lodz, Poland

Polyamidoamine (PAMAM) dendrimers are ones of the earliest synthesized types of dendrimers [1]. Like all dendrimers they are characterized by a globular shape, plenty of surface groups on the surface and empty internal cavities inside. This very characteristic structure has made them promising materials in various applications, including biomedical ones. Although using dendrimers as therapeutic agents is a fast growing field, there are still concerns about their toxicity and impact on biological systems. In our studies we checked hemolytic activity of PAMAM dendrimers (fourth, fifth and sixth generations). This is a continuation of our earlier work where lower generations were investigated [2]. We observed that hemolysis increased in a generation- and concentration-dependent manner. Additionally, we noticed that the longer the incubation time, the bigger the release of hemoglobin. Since dendrimers have a high affinity toward proteins [3] we checked if the presence of serum albumins in the incubation buffer had a protective effect against the disruption of erythrocyte membranes. Such obtained results were more relevant to physiological conditions.

[1] Tomalia, D.A., Baker, H., Dewald, J.R., Hall, M., Kallos, G., Martin, S., Roeck, J., Ryder, J. & Smith, P. (1985) *Polym. J.* **17**, 117-132

[2] Domanski, D. M., Klajnert, B. & Bryszewska, M. (2004) *Bioelectrochem.* **63**, 189-191

[3] Klajnert, B., Stanislawska, L., Bryszewska, M. & Palecz, B. (2003) *Biochim. Biophys. Acta* **1648**, 115-126

Abstracts

– Bionanomaterials & design –

P-598

Tagging protein for immobilisation using self-assemble monolayers (SAMs)

T. T. Le[1], C. P. Wilde[2], A. E. Cass[1]

[1]Institute of Biomedical engineering, Imperial College london, U.K., [2]Department of Chemistry, Imperial College London, U.K.

We describe here a method to immobilise proteins in a well-controlled manner as well as methods to determine the amount of the protein immobilised on the surfaces. The immobilisation method uses self-assembled monolayers (SAMs) terminated by a carboxyl group absorbed on a gold film. After formation of the monolayers on gold surface, the terminal carboxyl group was then modified with an NTA group that can hold Ni(II) tightly due to its tetravalent chelation. A His-tag protein can be immobilised on this surface by affinity interaction of histidines of the tag to the two vacant coordination sites on Ni-NTA which was adopted from the method of protein purification using an affinity tag. The amount of protein immobilised on the surfaces was determined by fluorescence spectroscopy taking advantages of reversible immobilisation of His-tag proteins on the surfaces and micro contact printing (μCP). In addition, using patterned SAMs made by μCP we also demonstrate that the His-tag protein binds specifically to SAMs terminated by a carboxyl group and resists SAMs terminated by a hydroxyl group. Studies with Green Fluorescent Protein (GFP) showed that the immobilised protein was kept intact since the fluorescent properties of the protein were remained.

P-599

In situ ATR FTIR monitoring of the 7-Carboxysilane monolayers formation on germanium substrate

J. Matijašević, N. Hassler, G. Reiter, U. P. Fringeli

Institute of Biophysical Chemistry, University of Vienna, Vienna, Austria

The development and optimization of biomimetic surfaces required for biosensors and medical assays are made more efficient by quantitatively monitoring the surface chemical reactions *in situ* by means of attenuated total reflection (ATR) FTIR spectroscopy. Single-beam-sample-reference (SBSR) ATR as well as modulated excitation (ME) techniques have been applied to get physico-chemical information on growth and structure of the surface layer. SBSR and ME methods result in optimum background compensation and signal-to-noise ratio. Surface modification was performed on a germanium multiple internal reflection element (Ge-MIRE). Activation of the surface resulted in free Ge-OH groups used for a spontaneous chemical reaction with *7*-octenyltrichlorosilane (OTCS) in toluene. Formation of Ge-O-Si bonds was enabled by hydrolization of $Si\text{-}Cl_3$ after partial elimination of a tightly bound thin water layer covering the MIRE. Unwanted side-reaction hydrolization of $Si\text{-}Cl_3$ in solution followed by polymerization paralleled this process. Steady growing of the OTCS layer to multilayer thickness with increasing time was observed in all experiments. Most unexpectedly, in some experiments the end-standing double bond of OTCS was found to be partly oxidized even after being exposed only to toluene, probably because of catalysis by sub-micrometer molecular sieve particles remaining in toluene after drying. Finally theoretical means are presented enabling the calculation of the spectrum of dissolved OTCS in toluene, a prerequisite for background compensation during *in situ* studies of the growing layer.

P-600

Characterisation and application of biopolymer based nanotubes

F. Mumm, P. Sikorski

Department of Physics, NTNU, Trondheim, Norway

Top-down fabrication methods of nanochannels for microfluidic systems typically require extensive production and alignment procedures usually conducted in a cleanroom environment. Bottom-up synthesis does, on the other hand, often lack the robustness, which is needed for the continuous operation of such systems.

We present a method to harvest stable, bottom-up synthesized, naturally occurring assemblies of biopolymer nanotubes from the spines of the bristleworm *Aphrodita Aculeate* (Sea Mouse). The spines contain arrays of thousands of parallel nanochannels, each of which with an inner diameter of about 200 nm and a length of up to 1 cm. This alignment also gives the spines their unique colourful appearance due to a photonic crystal effect.

The spines are made of a composite material containing protein and the biopolymer chitin. Element analysis data revealed a protein content of about 50 %. Alkaline treatment can be used to remove all protein without affecting the organization of the chitin network. This also converts some of the chitin on the surface to chitosan and, thus, introduces surface charges, which are necessary e.g. for electroosmotic flow.

We physically characterised the tubes using AFM, SEM, and TEM, isolated tube arrays of various sizes, and integrated them in basic microfluidic chips made of Polydimethylsiloxan to determine their flow through properties using fluorescence microscopy. The setup might serve as a tool in chromatography as it combines the functionality of small diameter nanotubes with a relatively high sample throughput due to the large amount of parallel channels.

P-601

Multivalent chelator heads for organization and manipulating proteins

A. Reichel[1], J. Strunk[1], S. Lata[1], M. Gavutis[1], I. Gregor[2], J. Enderline[2], J. Piehler[1]

[1]Institute of Biochemistry, J. W. Goethe University, Frankfurt am Main, Germany, [2]Institut für Biologische Informationsverarbeitung 1, Forschungszentrum, Jülich, Germany

Studying protein function requires suitable techniques for handling, organizing and modifying proteins on the molecular level without impairing their functional properties. We have addressed this problem by synthesizing chelator head groups with multiple NTA moieties (multivalent chelator heads, MCH), thus providing stable binding of histidine-tags by multivalent interactions straight on molecular level. The recognition of different his-tags by MCH was characterized by kinetic and thermodynamic analysis. Indeed stable and stoichiometric binding was observed, which could be switched by competing agents. Various conjugates of these MCH with fluorescence dyes, biotin and surfaces were synthesized and characterized in detail. We demonstrate the capabilities of these switchable biochemical tweezers in several applications: (i) site-directed, stable yet reversible immobilization of proteins on gold and glass-type surfaces; (ii) tethering of proteins in an oriented manner onto solid-supported lipid bilayers for studying lateral interaction kinetics of membrane-anchored proteins; (iii) fluorescence labeling of proteins *in vitro* for protein interaction analysis in solution, and on the surface of living cells. Using site-specifically fluorescence labeled cytokine receptor domains we established assays for probing conformational changes in solution and on surfaces. These examples demonstrate the broad applicability of MCH for single molecule applications.

Abstracts

– Bionanomaterials & design –

P-602

Nanostructured Polyelectrolyte-based System as a Toolbox for Metal Ions Detection

E. Ronzitti[2], V. Caorsi[1], A. Diaspro[1]
[1]LAMBS, MicroSCoBio, Department of Physics, University of Genoa, Genoa, Italy, [2]European School of Molecular Medicine, IFOM-IEO, University of Milan, Milan, Italy

The capability of certain heavy metal ions to induce fluorescence decrease by a quenching mechanism suggested us to design and build a sensor potentially tunable for different ions at different concentrations. In order to investigate fluorescence changes due to the presence of these quenchers we entrapped the fluorescence molecules in a polyelectrolyte nanostructured system, the nanocapsule. Nanocapsules are able to provide versatility, biocompatility and specificity to our sensor. To carry out quantitative analysis, the fluorescent nanocapsules are immersed in an uniform electrical field to induce a metal ions spatial gradient of concentration. Therefore, this sensing system allows to probe precisely the concentration of a specific metal ion (in this case copper ions) strictly related to fluorescence variations, recorded in real time through a confocal microscope. We will present a sensing system prototype version, in which preliminary characterization and results obtained show the potentialities of the system also envisaging in vivo cellular applications.

O-603

DNA origami: folding DNA to create arbitrary shapes and patterns

P. W. Rothemund
California Institute of Technology, Pasadena, California, U.S.A.

A key goal for bottom-up nanofabrication has been to generate structures whose complexity matches that achieved by top-down methods. Here I describe a method for folding long single strands of DNA into arbitrary two dimensional target shapes using a raster fill technique. Self-assembled in a one-pot reaction from the 7 kilobase genome of phage M13mp18 and more than 200 synthetic DNA strands, the shapes are roughly 100 nanometers in diameter and nearly 5 megadaltons in mass. Experimental shapes approximate target shapes, such as a 5-pointed star, with a resolution of 3.5 to 6 nanometer and may be decorated by arbitrary patterns at 6 nanometer resolution to form words or images.
Enabled by a program for laying out complicated designs and, utilizing inexpensive unpurified DNA strands, this method helps move DNA nanotechnology from the realm of research towards that of engineering. The ability to create arbitrary shapes provides a new route to the bottom-up nanofabrication of complex nano-scale devices and instruments. Physicists and materials scientists should be able to use DNA origami to arrange optical, electronic, and mechanical components into novel materials or even an integrated "nanolaboratory" of their choosing. Biologists may be able to use these structures to position proteins and other biomolecules in precise arrangements to study their coupling. Indeed these structures may be thought of as a versatile "nanobreadboard", a simple platform for creating arbitrary nanostructures.

P-604

Zinc-mediated oligomerisation of double-stranded DNA

Y. Santoso, L. Le Reste, A. N. Kapanidis
Department of Physics and IRC in Bionanotechnology, University of Oxford, Oxford, United Kingdom

Biomolecular wires can serve as central building blocks for nanoscale electronics. It has long been suggested that DNA can become such a wire, since it is characterized by programmable and facile self-assembly, ease of manipulation, and favourable dimensions.
However, DNA as found in nature is a poor electronic conductor; hence, modifications are needed to improve its conductive properties. One such modification involves treating canonical B-DNA with divalent metal cations (such as Zn2+) at alkaline pH (>8.5); it has been suggested that the cations replace the imino protons of thymine and guanine, creating a string of cations that assists transfer of electrons along the length of a single DNA molecule; this form of DNA was defined as M-DNA.
We use ensemble and single-molecule fluorescence spectroscopy, and gel electrophoresis to study the formation and properties of M-DNA. Specifically, we subject labeled double-stranded DNA to conditions that lead to M-DNA formation, and we monitor several processes (electron transfer, Förster resonance energy transfer and fluorescence quenching) through changes in fluorescence properties. Our results show that conditions that lead to M-DNA formation also promote intermolecular association and oligomerisation of DNA fragments in a zinc-dependent and reversible way. Preliminary results show that adjustment of conditions (e.g., Zn2+ concentration) can control the number of DNA molecules found in the oligomeric DNA complex. Our result suggest that conductive DNA may be obtained through controlled Zn-mediated DNA oligomerisation rather than Zn-mediated electron transfer within a single DNA molecule.

O-605

Spider silk – a basis for new materials

T. Scheibel
Department of Chemistry, Lehrstuhl für Biotechnologie, Technische Universität München, Lichtenbergstr. 4, 85747 Garching, Germany

Biological materials often exceed the characteristics and properties of man-made ones. One well-known example is spider silk. “Dragline” silks compose the frame and radii of spider webs and are intensely studied due to their superior mechanical properties such as strength and toughness. Manufacturing the proteins that form dragline silks encountered many problems in the past. We developed a bacterial expression system that allows mimicking the natural silks. However, our system can be further employed to engineer new silk-like proteins with additional chemical functions. Besides the protein fabrication, we have developed a spinning technique to produce spider threads closely resembling natural draglines. However, the recombinant silk proteins can additionally be processed into hydrogels, spheres, films, among other assembly forms. In summary, our system allows industrial-scale fermentation of silk-like proteins with “engineered” properties and assembly forms.The controlled design of silk proteins and their assembly forms will serve as a basis for new materials in a variety of medical, biological, or chemical applications.

Abstracts

– Bionanomaterials & design –

O-606

From lambda DNA grafted colloids with specific binding ends to finite size clusters.

T. Schmatko[1], B. Bozorgui[1], N. Geerts[1], D. Frenkel[1], E. Eiser[2], W. C. Poon[3]
[1]Fom institute AMOLF, Kruislaan 407 1098 SJ Amsterdam, NL, [2]Dpt of chemical engineering, University of Amsterdam, Nieuwe achtergracht 166, 1018 WV AMsterdam, NL, [3]School of physics, The University of Edingurgh, Mayfield Road, Edinburgh, EH93JZ, UK

The hybridisation of complementary DNA strands offers many possibilities for the use of this macromolecule in micro and nanotechnologies. Hence colloidal particles grafted with distinct short ss-DNA can bind specifically with each other via suitable DNA linkers. Such DNA coated colloids can be used as sensors for nucleotide polymorphism and may offer a route to novel colloidal crystals[1,2]. To date experiments with DNA colloid mixtures have used short DNA such as its polymeric potential has not been exploited. We report the 1st experimental investigation of the aggregation of particles bridged by long DNA[4]. We mixed 2 species of particles grafted with ds λ-DNA displaying short complementary ss overhangs as binding ends. Confocal microscopy showed the formation of size limited clusters. Simulations suggest that the close contact and the limitation to grow we observed both result from entropic exclusion of the bridging DNA from the space between particles.

1 R. Jin,G. Wu, Z. Li, C.Mirkin and G. C. Schatz, J. Am. Chem. Soc.,2003, 125, 1643.
2 P. H. Rogers, E. Michel, C. A. Bauer, S. Vanderet et al, Langmuir, 2005, 21, 5562.
3 4 T. Schmatko, B. Bozorgui, N.Geerts, D.Frenkel et al, Soft Matter, 2007, DOI : 10.1039/b618028k

P-607

Development of QCN nanobiosensor for the d etection of Plant Growth Promotory *Rhizobacteria*

K. P. Singh
Biophysics Laboratory, CBS&H, G.B.Pant University of agriculture & technology, Pantnagar-263145, India

Nanotechnology is playing an important role in the field of Biosensors. Functional nanoparticles bound to Biomolecules have been used in biosensors to detect and amplify the response signals. Nanoparticles are also be used to enhance the immobilized Biomolecules and lower the detection limit of Biosensors. Molecularly imprinted polymers (MIPs) represent a new class of materials that have artificially created receptor structures and are commercially applied in assays and sensors. In this presentation QCN biosensor for the detection Plant growth Promotory Rhizobacteria is explained with specific preparation of Core MIP with the help of core shell gold nanoparticles (SiO_2@Au) and its immobilization on gold coated AT cut quartz crystal by attaching the system for electronic circuit and frequency analyzer. The nanoparticles SiO2@Au is used as functional nanoparticles for immobilization of Antigen and raised antibody reaction for more specific and enhanced response on the QCN. This biosensor is more specific, selective and more sensitive due to high reactivity and binding affinity of nanoparticles and named Nanobiosensor. The detection of some more bacteria's and viruses also tested by this Nanobiosensor successfully.

O-608

Functional studies on membrane proteins using micro- and nano-BLMs

C. Steinem, E. K. Schmitt, D. Weiskopf
University of Goettingen, Institute for Organic and Biomolecular Chemistry, Germany

Peptides and proteins forming ion channels in lipid membranes are one of the major targets for the development of new drugs. Screening of new potential drugs for ion channels is, however, hampered by the lack of a chip-based screening system with high resistance and long-term stable membranes. Here, planar chip-based arrangements of pore-suspending lipid bilayers, termed micro- and nano-black lipid membranes (BLMs) based on highly ordered porous substrates will be discussed. Lipid membranes suspending nanometre- to micrometer-sized pores of highly ordered porous substrates were established that were characterized by means of electrical impedance spectroscopy revealing the formation of single lipid bilayers. Membrane resistances were in the order of Gigaohm. In contrast to classical BLMs, the membrane resistances of micro- and nano-BLMs decreased continuously with time. We hypothesised that membrane areas on the porous support are decoupled from each other by the thiol-submonolayer so that the entire membrane does not rupture in an all-or-none process. This proposed rupture process was visualized and confirmed by fluorescence microscopy. Moreover, by means of FRAP-experiments we were able to show that lipids in the pore-suspending part diffuse freely. The potential of the system to be suited for channel activity monitoring has been demonstrated by inserting various peptides such as gramicidin, alamethicin and the transmembrane domain of the HIV-1 accessory peptide Vpu_{1-32}. Even large proteins can be inserted as was demonstrated by the channel protein OmpF from *E. coli.*

P-609

Can mitochondria be utilized as nanolasers?

R. Thar
Institute of Biology, University of Copenhagen, Denmark

Most mitochondria in eukaryotic cells show a filamentous morphology with typical lengths of 5-10 μm. They are aligned with the microtubules of the cytoskeleton, forming a complex microtubules/mitochondria-network. Microtubules and mitochondria possess a higher optical refractive index than the surrounding cytoplasm. Thus, their network can in principle act like fiber-optic cables, i.e. it should efficiently guide electromagnetic radiation. The internal membrane structure of filamentous mitochondria is characterized by highly organized infoldings, yielding to a cross-striated structure consisting of layers with alternating refractive indices, similar to multi-layer-coatings utilized in technical interference mirrors. We present analytical and simulational studies, demonstrating that the complex inner structure of filamentous mitochondria resembles optical resonators already being utilized in technical distributed-feedback lasers. This property might be in the future exploited for the developement of "bionanolasers" based on filamentous mitochondria. The energy supply for such "bionanolasers" would be based on organic substrates, which are respired in the mitochondrial respiration chain leading to the production of ATP. It is discussed, how this ATP-production can be used to generate excited fluorescent molecules (the "laser dye") inside the mitochondria. When reaching the lasing threshold, both ends of such filamentous mitochondria will emit coherent light. The combination of such "bionanolasers" with complex microtubules-networks (fiber-optic cables) might open unforeseeable possibilities like "nanophotonics circuits" or even quantum computers.

Abstracts

– Bionanomaterials & design –

P-610

Molecular design of bioactive compounds and nanostructures for targeted delivery

Y. Tourleigh, D. Golik, K. Shaitan

Bioengineering Dpt., Lomonosov Moscow State Univ., Russia

Molecular dynamics (MD) approach employing an all-atom force field and special procedures was used to model and design nanocontainers for inclusion of biocompounds and delivery through lipid membrane. Carbon nanotubes may serve as non-immunogenic containers of biocompounds and vehicles delivery to the cell membrane. These two properties – inclusion and transportation – are interconnected and establish the main challenge in nanovehicle design. MD simulation of systems containing a carbon nanotube, water, a lipid bilayer, and a molecule to be delivered is a way to explore the problem.

Release of the peptide was performed by expulsing it in various media, including the lipid membrane. A set of swelling model spheres emulated the releasing compound (it is supposed that some external signal such as light is needed for the activation of release). For this purpose a modified variant of steered molecular dynamics (SMD) was developed. The conformational state of the peptide was studied in view of chemical stability of the substance under the shock action. The initial helical conformation deformed most greatly at ejection of the polypeptide into vacuum and least of all – into the membrane. Apparently, the environment plays a deforming and structure-forming role in this process. Conformational changes of the polypetide molecule are reduced with a decrease of the swelling rate.

The work was supported by RF Federal Agency on Science and Innovation, RF Federal Agency on Education, Russian Foundation for Basic Research (projects no. 06-04-08136, 07-04-01169) and US CRDF (2803).

P-611

DNA nanostructures and molecular motors

A. J. Turberfield, J. Bath, J. Malo, R. P. Goodman, H. M. Carstairs, C. M. Erben, A. J. Genot, D. N. Selmi

University of Oxford, Department of Physics, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, U.K.

DNA is a wonderful material for nanoscale construction. It is a structural material whose self-assembly can be programmed by making use of information stored in its base sequence. DNA hybridization can also be used as an energy source for molecular devices. We describe our recent work on self-assembled DNA polyhedra [1] and their applications in three-dimensional nanofabrication and as molecular cages [2] and our progress towards the construction of a free-running synthetic molecular motor fuelled by DNA [3].

[1] R. P. Goodman *et al.*, *Science* **310**, 1661-1665 (2005).

[2] C. M. Erben, R. P. Goodman, A. J Turberfield, *Angew. Chem. Int. Ed* ***45***, 7414-7417 (20006).

[3] B. Yurke *et al.*, *Nature* **406**, 605-608 (2000); A. J. Turberfield *et al.,Phys. Rev. Lett.* **90**, 118102 (2003); P. Yin *et al., Angew. Chem. Int. Ed.* **43**, 4906-4911 (2004); J. Bath, S. J. Green, and A. J. Turberfield, *Angew. Chem. Int. Ed*. **44**, 4358-4361 (2005); S. J. Green, D. Lubrich and A. J Turberfield, *Biophys. J.* **91**, 2966-2975 (2006).

P-612

The influence of monomer geometry on the structure of self-assembled porphyrin nanoaggregates

J. Valanciunaite[1], V. Poderys[1], R. Augulis[2], M. Barkauskas[1], M. Vengris[1], S. Bagdonas[1], R. Rotomskis[1]

[1]Vilnius University Laser Research Center, Sauletekio 9 c3, LT-10222, Vilnius, Lithuania, [2]University of Groningen, Nijenborgh 4, Groningen 9747AG, The Netherlands

J-aggregates of organic dyes attract much scientific interest due to unique nonlinear optical properties resulting from their highly ordered structure. Water soluble meso-tetra(4-sulfonatophenyl)porphine ($TPPS_4$) spontaneously forms J-aggregates in aqueous solutions at low pH and/or at high ionic strength. Moreover, it was shown recently that in highly concentrated acidic solutions $TPPS_4$ molecules self-assemble into complex tube-like nanostructures, exhibiting absorption bands characteristic for J- and H-aggregates. However, despite the extensive studies, the understanding of morphology of $TPPS_4$ nanoaggregates at molecular level remains fragmentary. In this study, we investigated meso-tetraphenylporphine sulfonates (TPPS) with different number and positions of sulfonato side groups to determine the relationship between the chemical structure of the monomer and the resulting geometry of nanoaggregates. The investigation was done by comparing structural and spectroscopic data of different nanoaggregates, collected using atomic force microscopy (AFM) and flow-induced linear dichroism (FILD) methods. LD spectra were obtained for $TPPS_4$, $TPPS_3$, and $TPPS_{2a}$ aggregate solutions, while for $TPPS_{2o}$ no detectable LD was observed. This finding was supported by AFM data, which showed that cylindrical nanoaggregates formed in all cases except $TPPS_{2o}$. Based on this data, a preliminary structural model of TPPS nanoaggregates is proposed.

Abstracts

– Fluorescent proteins: applications –

O-613

Complexities of green fluorescent proteins as probes in biological systems.

C. R. Bagshaw
Department of Biochemistry, university of Leicester, U.K.

Green fluorescent protein and it variants have complex photophysical properties that are evident on time scales from femtoseconds to hours. This presentation will review the interrelated properties of protonation, photochromism and dimerisation. In particular we have identified a proton-coupled isomerisation that occurs on the millisecond to seconds time scale, depending on the precise variant, that is responsible for "blinking" of single fluorophores. These findings, along with reversible photobleaching reactions and rapid excited-state proton transfer reactions, have implications for the use of these probes in Förster energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) measurements.

P-614

Polarized stimulated emission depletion in the EGFP chromophore

A. J. Bain[1], T. A. Masters[1], R. J. Marsh[1], D. A. Armoogum[1], B. Larijani[2]
[1]Department of Physics and Astronomy, University College London, United Kingdom, [2]Cell Biophysics Laboratory, Cancer Research UK London Research Institute, United Kingdom

Stimulated emission depletion (STED) population and polarisation dynamics are used together with time-resolved fluorescence anisotropy measurements to determine the full alignment of EGFP chromophores following two-photon excitation at 800nm. Conventional fluorescence techniques do not provide this information and as molecular alignment plays a significant role in biophysically important processes such as resonance energy transfer, the determination of 'hidden' degrees of molecular order created by multiphoton excitation is highly desirable. Time-resolved linearly and circularly polarised two-photon fluorescence anisotropy measurements are used to determine the structure of the transition tensor in EGFP, and are combined with STED intensity and anisotropy measurements to yield the hitherto 'hidden' degrees of molecular order created by two-photon excitation. The efficiency of STED in molecular systems is governed by the interplay between the STED cross section, molecular alignment and the rate of vibrational relaxation in the ground state. Vibrational relaxation dynamics in the ground electronic state of EGFP are investigated using shaped picosecond and femtosecond depletion pulses. EGFP is seen to possess a significant STED cross section (ca. $2x10^{-16}cm^2$), an extremely high degree of initial molecular alignment and efficient vibrational relaxation (tvib ca. 500fs).

P-615

Tat-mediated transport of folded proteins across an ion-tight membrane

Y. Bollen, H. Lill
Vrije Universiteit Amsterdam, Department of Structural Biology, Amsterdam, The Netherlands

Biological membranes separate cells from their external environment. The membranes keep wanted molecules inside and unwanted molecules outside, yet at the same time they allow for the controlled exchange of matter and information between the cell and its surroundings. One of the most striking examples of the paradoxical functionality of a membrane is the twin arginine translocation (Tat) system. The system transports specific proteins in their folded conformation across the bacterial cytoplasmic membrane while keeping it ion-tight. The Tat-system has been studied extensively by us and many others, mainly by molecular biological and biochemical techniques. Based on these indirect data a mechanism for Tat-transport has been proposed. During the last five years no significant progress has been made in understanding the mechanism by which the Tat system performs its intriguing task. We aim to study Tat-mediated protein transport using single-particle tracking in a fluorescence microscope. The study will be done in living bacteria, because the activity of reconstituted Tat systems is very low. Genetic fusions will be made between the main components of the Tat system and the fluorescent proteins eGFP and mCherry. An artificial fluorescent substrate of adjustable size will also be made, consisting of a small chain of eGFP molecules fused to a Tat-specific signal peptide.

P-616

Mapping distances and relative orientations of yellow cameleon domains through quantitative FRET analysis

J. Borst[1], S. Laptenok[1], A. Westphal[1], N. V. Visser[2], J. Aker[1], A. van Hoek[2], A. J. Visser[1]
[1]Microspectroscopy Centre, Laboratory of Biochemistry, Wageningen University, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands, [2]Microspectroscopy Centre, Laboratory of Biophysics, Wageningen University, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands

Förster Resonance Energy Transfer (FRET) is a widely used method for monitoring interactions between or within biological macromolecules conjugated with suitable donor-acceptor pairs. Donor fluorescence lifetimes in absence and presence of acceptor molecules are often measured for the observation of FRET. However, these lifetimes may originate from interacting and non-interacting molecules, which hampers quantitative interpretation of FRET data. Here we describe a methodology for the detection of FRET that monitors the rise time of acceptor fluorescence upon donor excitation thereby detecting only those molecules undergoing FRET. Subsequently,, the relative orientation between donor and acceptor chromophores is obtained from time-dependent acceptor fluorescence anisotropy measurements. The calcium sensor Yellow Cameleon 3.60 (YC3.60) was chosen because it changes its conformation upon calcium binding, thereby increasing the FRET efficiency between the fluorophores. After mapping distances and orientation angles between the FRET moieties in YC3.60, a structure of this FRET sensor could be modelled. This spectroscopic approach presents a rapid and correct methodology to model structural changes in a protein upon ligand binding.

Abstracts

– Fluorescent proteins: applications –

P-617

Possible folding aid mechanism of alginate: effect on bovine carbonic anhydrase refolding

M. A. Esmaeili, R. Yazdanparast
Institute of Biochemistry and Biophysics, P.O. Box 13145-1384, University of Tehran, Tehran, Iran

Protecting proteins from aggregation is one of the most important issues in both protein science and protein engineering. In this research, we have used bovine carbonic anhydrase as a model to understand the mechanism of aggregation during refolding and its prevention by using alginate, a linear polysaccharide. Based on literature, removal of the metal ion from the bovine carbonic anhydrase by a chelator such as EDTA enhances the propensity of the enzyme to adopt the molten-globule state. In this study, we found that alginate binds to this state of the enzyme and prevents its aggregation. The reactivation and refolding kinetics indicated that the refolding rate constant of the first refolding intermediate (I_1) to the second one (I_2) is promoted by the addition of alginate. Fluorescence quenching studies further indicated that alginate could bind to the aggregation-prone species I_1, resulting in the protection of the exposed hydrophobic surfaces, minimization of the protein surface, and more importantly, enhancing the refolding rate of I_1. Among the various alginate concentrations used, it was found that the best refolding yield was obtained at an alginate concentration of 2.5 % (w/v). A good correlation between the refolding yield and the suppression of protein aggregation by alginate at this concentration was also observed. According to Dong classification of folding aids, alginate belongs to the folding aids that stabilize the unfolded proteins or folding intermediates at appropriate concentrations. These results suggest that alginate works by a mechanism similar to the well established mechanisms used by chaperones and chemical promoters.

O-618

Ultrafast excited-state dynamics of the photoswitchable protein Dronpa

E. Fron[1], C. Flors[1], G. Schweitzer[1], S. Habuchi[1], H. Mizuno[2], R. Ando[2], A. Miyawaki[2], F. C. de Schryver[1], J. Hofkens[1]
[1]Katholieke Universiteit Leuven, Belgium, [2]RIKEN Brain Science Institute, Wako city, Japan

Dronpa is a photoswitchable protein from the family of green fluorescent proteins (GFPs).[1–3] Photoswitching is thought to involve forward and backward proton transfer between a bright deprotonated form (B) and a dark protonated form (A_2). We have used femtosecond transient absorption spectroscopy to determine the kinetics of the first step of photoconversion, which we have measured as 4 ps. The assignment of this time constant has been supported by comparison with a pH-induced protonated sample at pH 5 (A_1). The 2-fold isotope effect on the kinetics of this process shows that excited-state proton transfer (ESPT) is involved in this step, and its lower value as compared to GFP points to a different proton relay mechanism. Furthermore, we also confirm that the acid induced protonated form A_1 and the photoconverted protonated form A_2 are two distinct spectroscopic species.

1. Ando, R.; Mizuno, H.; Miyawaki, A., *Science* **2004**, *306*, 1370.
2. Habuchi, S.; Ando, R.; Dedecker, P.; Verheijen, W.; Mizuno, H.; Miyawaki, A.; Hofkens, J. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9511-9516.
3. Fron, E.; Flors, C.; Schweitzer, G.; Habuchi, S.; Mizuno, H. ; Ando, R.; Miyawaki, A.; De Schryver, F.C.; Hofkens J. *J. Am. Chem. Soc.* **2007**, doi 10.1021/ja069365v, in press

P-619

The adherent chemokine FKN is oligomeric. Evidences from biophysical experiments (FRAPP and BRET).

J.-E. Guet[1], P. Deterre[2], P. Hermand[2], F. Pincet[1]
[1]Laboratoire de Physique Statistique, ENS, CNRS UMR8550, Paris, France, [2]INSERM U543, Université Pierre et Marie Curie, Faculté de Médecine, Paris, France

Fractalkine (FKN) is a membranous chemokine of 90kDa composed of a cytoplasmic tail, a transmembrane α-helix (TM), a long mucin like stalk, and a chemokine domain. With its unique known receptor, CX3CR1, it is involved in cell adherence. In order to determine the oligomerization status of FKN and to show its possible interaction with cytoskeleton, we analyzed by FRAPP (Fluorescence Recovery After Pattern Photobleaching) its diffusion in living cell membrane. Results show that FKN diffuses very slowly ($0.02\mu m^2/s$) as compared with CX3CR1 and CCR5 which show a 8 times higher diffusion rate despite a larger transmembrane radius (at least 7 α-helix). Moreover, this result is not modified by the deletion of the cytoplasmic tail. Hence, the slow diffusion of the FKN molecule is not due to its anchoring to the cytoskeleton. Moreover, the deletion of the mucin stalk considerably results in an increased lateral diffusion rate, suggesting that the highly glycosylated stalk contributes to the slow diffusion of FKN. Most probably, it is mainly caused by oligomerization. BRET (Bioluminescence Resonance Energy Transfer) experiments made in the lab reinforce this conclusion and showed that the FKN aggregation occurs through the TM domain. Accordingly, a construct of FKN mutated in the TM domain showed a diffusion rate significantly higher than FKN ($0.05\mu m^2/s$). Altogether the BRET and FRAPP data converge in showing that FKN is an oligomer with a high degree of oligomerization.

P-620

Lateral resolution improvement in fluorescence microscopy by structured illumination

L. Hirvonen, O. Mandula, K. Wicker, R. Heintzmann
Randall Division of Cell and Molecular Biophysics, King´s College London, U.K.

Fluorescence microscopy is an important tool in biological imaging, allowing non-destructive imaging of specific components inside living cells and tissues. Unfortunately, due to diffraction phenomenon, the resolution is limited to about 200 nm in lateral direction. Resolution improvement by a factor of two can be achieved using structured illumination, where a fine grating is projected onto the sample, and the final image is reconstructed from a set of images taken at different grating positions.

Further resolution improvement can be achieved by saturating the excited state of the fluorophores, but so far the required high illumination intensities have not been compatible with live cell imaging. Recently discovered photoswitchable proteins that undergo transitions that are saturable at low illumination intensities could solve this problem. Combining this concept with structured illumination, theoretically unlimited resolution can be achieved, where the smallest resolvable distance will be determined by signal-to-noise ratio. The concept is illustrated, and first experimental results are shown.

Abstracts

– Fluorescent proteins: applications –

O-621

Excited state proton transfer in the green fluorescent protein and its mutants

D. Huppert[1], P. Leiderman[1], L. Genosar[1], L. Cohen[2]

[1]Raymond and Beverly Sackler Faculty of Exact Sciences, School of Chemistry, [2]G. Wise Faculty of life science, Tel Aviv University, Tel Aviv 69978, Israel

Proton transfer reactions are among the most common and important chemical and biological processes in aqueous solutions. We used the accumulated knowledge of ESPT processes in solution to study, by time-resolved spectroscopy several, aspects of the excited state proton transfer of wild type GFP (wt-GFP), GFP mutants and synthetic analogs of the GFP chromophore.
The main topics we shall discuss in my talk:
a) The temperature dependence of the time-resolved emission of wt-GFP shows that the proton transfer rate slows down as the temperatures decreases. At very low temperatures, T < 80K, the proton transfer rate is almost independent of temperature. We explain the temperature dependence of the proton transfer rate by a model that includes an intermolecular vibration assisted tunneling.
b) We have found that at relatively low hydrostatic pressures (P < 0.8 GPa), the time-resolved emission of the protonated form, ROH of wt-GFP, is only slightly modified as the pressure increases.
c) We have studied several mutants that strongly affect the proton transfer rate. Among them E222D, T203V, S205V and T203/S205V.
d) We have used short wavelength pulses at 270 – 320 nm to excite wt-GFP. We have found that the ESPT rates slow by a factor of 10 and 3 for H_2O and D_2O samples, respectively.

P-622

Fluorescence lifetime imaging applied to molecular motors in muscle fibres

D. Ibanez-Garcia[1], P. M. Lanigan[1], J. Eccleston[2], M. R. Webb[2], J. Requejo-Isidro[1], E. Ausorius[1], M. A. Neil[1], P. M. French[1], M. A. Ferenczi[1]

[1]Imperial College London, U.K., [2]NIMR, Mill Hill, London, U.K.

During muscle contraction, the rate constants controlling the biochemical steps in the actomyosin ATPase, change according to the force exerted on, or by, the muscle. We investigate the use of Fluorescence Lifetime Imaging (FLIM) of fluorescently labelled ATP analogues to probe changes in the myosin nucleotide binding pocket environment which may provide information about the ATP hydrolysis kinetics and its strain dependence. FLIM measurements were carried out in permeabilized muscle fibres from rabbit psoas in the presence of low concentrations of a coumarin-labelled ATP analogue (DEAC-pda-ATP), where the dye is attached to the 3'-position of the ribose of the nucleotide via a 3-carbon flexible linker. The lifetime and fluorescence properties of this analogue are altered upon interaction with a muscle fibre. FLIM images of stretch muscle fibres, show clear difference in lifetime between the I-band and the Overlap (ADP-actomyosin complex) and Non-overlap (ADP-myosin complex) regions in the A-band. Multiexponential analysis of the fluorescence lifetime decays reveal that the lifetime of the nucleotide changes depending on whether the nucleotide is free in solution (0.64 ns), bound to actin (3.10 ns), or bound to myosin (1.59 ns) or actomyosin (1.20 ns) in the ADP form. This novel application of FLIM to muscle fibres, and the sensitivity of the analogue to changes in the microenvironment, provides a potential use of FLIM to study changes in the actomyosin complex during force generation steps in muscle.

P-623

Phototransformation of autofluorescent proteins in fluorescence microscopy

G. Jung
Saarland University, Biophysical Chemistry, Building B2.2, 66123 Saarbruecken, Germany

Phototransformations of autofluorescent proteins find application in high resolution microscopy and in studying cellular transport mechanisms, but they are found bothersome when accidentally occurring in blinking or photobleaching (BL). Here, we present data on phototransformations of a photoactivatable GFP in confocal microscopy. Photoconversion (PC) is achieved solely by excitation of the barely absorbing anionic chromophore state R_{eq}^- in the GFP mutant Thr203Val. Besides the well-known shift of the equilibrium between the neutral chromophore state RH and R_{eq}^-, the photoconverted anionic chromophore R_{pc}^- exhibits a reduced fluorescence lifetime of 2.2 ns. The fluorescence lifetime which is measured in fluorescence lifetime imaging microscopy with spatial resolution depends however on the excitation conditions and history. The underlying photochemistry is described by the kinetic scheme of consecutive reactions, $R_{eq}^- > R_{pc}^- > P_{dark}$, in which the anionic chromophore species and the dark protein P_{dark} are coupled by PC and BL. Time correlated single photon counting in a confocal geometry of diffusing species is used to compute the quantum yields for PC and BL for the anionic chromophore species, Φ_{pc} and Φ_{bl}. The assessed values are $\Phi_{pc} = 5.5*10^{-4}$ and $\Phi_{bl} > 1*10^{-5}$. Based on our interpretation, PC is responsible for spectroscopic peculiarities of autofluorescent proteins in confocal microscopy [1]. Furthermore, previous interpretations of photodynamics on the single molecule level have to be reconsidered.
[1] Jung, G., Werner, M., Schneider, M., *submitted*.

O-624

Comparative FCS- and TCSPC studies on autofluorescent proteins

G. Jung[2], A. Zumbusch[1]

[1]Department Chemie, Universität Konstanz, D-78457 Konstanz, Germany, [2]Biophysikalische Chemie, Universität des Saarlandes, Saarbrüken, Germany

Autofluorescent proteins have found widespread use in confocal microscopy as genetically encodeable markers which fluoresce without the addition of external cofactors. The most prominent example is the green fluorescent protein (GFP) which can be detected in live cells with sensitivities down to the single molecule level. A good knowledge of the photophysical properties of GFP is of uttermost importance for its efficient use as a label as well as for the development of new mutants with altered spectroscopical characteristics. Here we present a comparative study of the influence of mutations of three amino acids which crucially determine the photophysical behavior of the GFP. Our data highlight the influence of the protein's hydrogen-bonding network on the equilibrium between the different chromophore states and on the efficiency of the excited state proton transfer (ESPT). The mutagenic approach allows us to separate different mechanisms responsible for fluorescence quenching, some of which were previously discussed theoretically. The results have implications for the development of new strategies for the generation of GFP species with specific photophysical properties. One example presented here is an uncommon blue fluorescing variant.

Abstracts

– Fluorescent proteins: applications –

P-625

Fluorescence lifetime imaging microscopy of myosin essential light chain in skeletal muscle fibres

D. S. Ushakov, A. Konitsiotis, D. Ibanez-Garcia, T. G. West, E. Auksorius, J. R. Isidro, P. M. French, M. A. Ferenczi
Imperial College London, U.K.

The contraction of muscles is driven by the ATP-dependent interaction of actin and myosin filaments. Fluorescence lifetime imaging microscopy (FLIM) of a probe attached to myosin may provide information about the changes in its conformation upon ATP hydrolysis and steps involved in force generation. To investigate this we expressed human myosin essential light chain (ELC) bearing a single cysteine residue near the C-terminus (Cys-178), which was labelled with thiol-reactive coumarin. The light chain was introduced in rabbit *psoas* muscle fibres where it was exchanged for the native ELC. Confocal microscopy revealed that the ELC was present only in the A-bands of muscle fibres, confirming the specificity of the exchange. The properties of treated fibres were examined by isometric force measurements. The fibres undergoing exchange treatment without any exogenous ELC produced 50% less force, while the addition of fluorescently labelled light chain restored the force to 85-90%. The FLIM study of ELC-coumarin in relaxed fibres showed a double exponential fluorescence decay with 2.93 nsec and 0.95 nsec components. In rigor fibres both components decreased to 2.61 nsec and 0.63 nsec, respectively, approaching those of ELC-coumarin free in solution (2.21 nsec and 0.54 nsec). These data suggest a nucleotide-dependent conformational change in vicinity of the C-terminal lobe of ELC. Supported by the IRC in Bionanotechnology.

O-626

High resolution structural details of functional dynamics in the Green Fluorescent Protein

J. J. van Thor[1], K. Ronayne[2], M. Towrie[2], J. T. Sage[3]
[1]Imperial College London, Wolfson Laboratories, Molecular Biosciences Division, London SW7 2AZ, U.K., [2]Central Laser Facility, Council for the Central Laboratory of the Research Councils, Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire, U.K, [3]The Department of Physics and Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston, Massachusetts 02115, U.S.A.

We present high resolution details of structural dynamics in the photocycle of Green Fluorescent Protein, from ultrafast mid-infrared spectroscopy in combination with X-ray crystallographic determination of cryogenically trapped reaction intermediates. The fluorescence photocycle of the wild type *Aequorea victoria* Green Fluorescent Protein (GFP) involves Excited State Proton Transfer (ESPT), which causes destabilising electrostatic rearrangements on a picosecond time scale. I shall discuss X-ray crystallographic structure determination of the cryogenically trapped reaction intermediates, optical and cryo-FTIR spectroscopy and ultrafast mid-infrared spectroscopy of the phototransformation and fluorescence photocycle reactions respectively, providing an in depth analysis of the vibrational and dynamical response to light-induced electrostatics changes and the significance of the observation of ultrafast and low barrier motions.

P-627

Automatic phase corrections in structured illumination microscopy

K. Wicker, O. Mandula, L. Hirvonen, R. Heintzmann
Randall Division of Cell and Molecular Biophysics, King´s College London, U.K.

Diffraction limits the resolution of a light microscope to about 200 nm (Abbe limit). In the last years several methods have been introduced which exploit nonlinearities in order to circumvent the Abbe limit and achieve better resolution. As a wide-field method Structured Illumination Microscopy (SIM) has the advantage of relatively fast data acquisition. However, it requires intensive computation for the reconstruction of images from the acquired data. The quality of the reconstructed images is decreased by aberrations as well as phase and amplitude uncertainties in the illumination, which must be accounted for in the reconstruction algorithms.
We present a reconstruction method which works in the presence of unknown phase and amplitude fluctuations in the excitation pattern.

Abstracts

– Chemotaxis and signal transduction –

O-628

Biophysics of chemotaxis in sperm

L. Alvarez, B. U. Kaupp, L. Dai
Institute of Neurosciences and Biophysics, Forschungszentrum Jülich, Germany

Chemotaxis of sperm is well established in sea urchins. In the species *Arbacia punctulata*, the chemoattractant released by the egg and its cognate receptor on the sperm surface has been identified. Stimulation of sea urchin sperm by the chemoattractant triggers the production of intracellular cyclic GMP (cGMP) which acts as a second messenger. Cyclic GMP induces a cascade of intracellular reactions leading to an increase of intracellular [Ca^{2+}] and a motor response. Our research is focused on the molecules and mechanisms by which Ca^{2+} enters the cell and how changes in [Ca^{2+}] determine the motor response of sperm and the navigation pattern in a gradient of chemoattractant.

P-629

Polarity and proticity details of the nitroxide micro-environment in spin labeled proteins

E. Bordignon, H. Brutlach, L. Urban, J. P. Klare, H.-J. Steinhoff
Fachbereich Physik, Universität Osnabrück, Barbarastrasse 7, 49076 Osnabrück, Germany

Taking advantage of the improved spectral resolution of high-field EPR at 95 GHz/3.4 T as compared to conventional X-band EPR (9.5 GHz/0.34 T), detailed information on the nitroxide micro-environment in spin labeled proteins can be obtained.
Nitroxide spin label side chains are introduced at positions 88 to 100 in the AS-1 sequence of the membrane adjacent HAMP domain of the transducer protein, NpHtrII, which is reconstituted in complex with sensory rhodopsin, NpSRII. Position dependent variations of the values of the nitroxide magnetic tensor components g_{xx} and A_{zz} suggest that the spin label side chains at positions 88 to 93 of AS-1 are located between a hydrophobic and a hydrophilic micro-environment. The observed periodicity of the polarity properties of the respective spin label micro-environment agrees with an alpha-helical secondary structure of this part of AS-1 and validates a recently published molecular model (1). Comparison between the polarity data obtained at low temperature for positions 95 to 100 of AS-1 and the water accessibility data obtained at room temperature will provide evidence for the structural changes occurring in dynamic domains of protein upon freezing. This study also aims to investigate the g_{xx} heterogeneity resolved in the W-band spectra of specific spin labeled positions in proteins in terms of heterogeneity of nitroxide populations characterized by different degree of H-bonding to the NO group. The data are compared with theoretical calculations available in literature.
1. Brutlach et al. (2006) *Applied Magnetic Resonance* 30, 359-372

P-630

Kinase/phosphatase control of Ca2+-dependent smooth muscle contraction

M. Brumen, A. Fajmut
Institute Jozef Stefan, Ljubljana; University of Maribor, Dept. of Physics & Dept. of Biophysics, Maribor, Slovenia

Smooth muscle contraction is initiated by Ca^{2+}/calmodulin (CaM) dependent activation of myosin light chain kinase (MLCK) which with the phosphatase (MLCP) controls the net extent of phosphorylated MLC, that enables cross-bridges to cycle. In our recent mathematical model of a detailed kinetic scheme describing interactions among Ca^{2+}, CaM and MLCK with eight states we predicted the amount of MLCK active form - Ca_4CaM·MLCK. Here we expanded the existing well known 4-state latch-bridge model, coupled it with the above mentioned model, and modelled the reactions of phosphorylation and dephosphorylation of MLC by the M-M kinetics. This approach enables the prediction of Ca^{2+}-dependent velocities of phosphorylation and dephosphorylation. Furthermore, the sensitivity of main system variables like MLC phosphorylation and velocities of phosphorylation/dephosphorylation, is studied by the control theory. Generally, kinases suppose to control the amplitudes more than the duration of a signal whereas phosphatases tend to control both. We show that in the contractile apparatus MLCK and MLCP control phosphorylation in that manner. Furthermore, the influence of elevated total concentration of MLCK, the property of bronchial muscle cells of asthmatic subjects, is studied. We predict by the model that elevated total MLCK concentration affects the magnitude of MLC phosphorylation and, hence, elevates the magnitude of force. We show also that an inherent dynamical property of the system leads to almost stationary value of force on the cellular level even in the case when Ca^{2+} oscillates.

P-631

A puzzling photobehaviour in two different strains of Halobacterium salinarum

N. Deiana[1], G. Cercignani[2], S. Lucia[1]
[1]Istituto di Biofisica CNR, Pisa, Italy, [2]Dipartimento di Biologia, Univ. di Pisa, Italy

The photobehaviour of *Halobacterium salinarum* depends on the light stimulation: switching off an orange light induces the reversal; switching on a green light, or a blue light against a constant orange background, causes a reversal frequency increase, which is depressed by switching off the same lights. We measured the photoresponses at different growth phases on cultures of two strains of the *H. salinarum:* NRC1 wild-type strain and its mutant Flx15 (BR^-, HR^-). We obtained photoresponses to 1-s blue pulses even in the absence of an orange background and the dose-effect curves to blue pulses both in the presence and in the absence of an orange background do not show appreciable differences. The responses to green or blue pulses are maintained over the various phases of the cell growth curve and the dose-effect curves show higher photosensitivity to blue light than that to green light, suggesting that the photoresponses to blue-light cannot be accounted for by SRII alone. In our conditions, an orange step-down doesn't induce any response, but if we use it as a prestimulus followed by a pulse, it affects the response to the pulse. This influence varies quantitatively and in sign depending both on culture age and on the wavelength of the light pulse. The results obtained up to date on Flx15 and their overall pattern, which is comparable to that obtained on NRC1, pose some problems in interpreting the mechanism(s) underlying the photoresponses and suggest that they might be accounted for by photosensing/transducing molecular sets different from those up to now ascertained in this archaeon.

Abstracts

– Chemotaxis and signal transduction –

O-632

Theoretical description of chemotaxis in sperm

B. M. Friedrich, F. Jülicher
Max-Planck-Insitute for the Physics of Complex Systems, Dresden, Germany

Sperm cells swim towards the egg propelled by a flagellum which beats regularly. In many species sperm show chemotaxis, i.e. they move upwards a gradient of chemoattractant molecules released by the egg.
Based on recent experiments on sea urchin sperm which indicate that the geometry of swimming trajectories is controlled by a signaling system in the sperm flagellum [1], we present a theoretical description of sperm chemotaxis.
We discuss swimming trajectories in two and three dimensions in the presence of a chemoattractant source. From this discussion, we derive the necessary properties of the signaling system which ensure reliable motion towards the source.
[1] B. Kaupp et al.: NCB 5,109 (2003)

P-633

Analysis of light-induced conformational changes of NpSRII-NpHtrII by time resolved and pulsed EPR

J. Holterhues[1], E. Bordignon[1], J. P. Klare[1], S. Martell[2], M. Engelhard[2], H.-J. Steinhoff[1]
[1]University Osnabrueck, Germany, [2]MPI for Molecular Physiology, Germany

The phototaxis receptor sensory rhodopsin II (SRII) mediates the photophobic response of the haloarchaeon *N. pharaonis* by modulating its swimming behavior. After excitation by blue-green light *Np*SRII triggers, by means of a tightly bound transducer molecule (*Np*HtrII), a signal transduction chain homologous to the two-component system in eubacterial chemotaxis.
Previous site directed spin labeling EPR studies performed on the complex *Np*SRII/*Np*HtrII$_{157}$ reconstituted in purple membrane lipids showed that light activation induces a displacement of the cytoplasmic edge of helix F. This rearrangement appears within the transition from the M1 to the M2 state and is sustained until the reisomerization of the retinal to the all-trans conformation. The structural modification of the receptor induces a rotation of helix TM2 in *Np*HtrII.
Time resolved EPR data obtained on selected positions in the cytoplasmic moiety of the receptor (helices B, C, F and G) and in the transducer (helix TM2 and HAMP domain) give insight into the light induced conformational changes. Distance changes upon light activation are observed via low temperature cw and pulsed EPR spectroscopy for the F helix in the receptor and for the first time in the HAMP domain of the transducer. The signal transfer mechanism from the receptor to the transducer will be discussed.

P-634

Differentiation of PC12 neuronal cells on chemically modified surfaces and in a NGF free medium

G. Lamour, N. Journiac, O. Friaa, G. Louis, A. Hamraoui
Laboratoire de Neuro-Physique Cellulaire, EA3817, Université René Descartes, Paris, France

Modified glass surfaces have been used to observe the extent to which local gradients of surface energy can stimulate the neurite outgrowth of adrenal pheochromocytoma cells (PC12) without addition of Nerve Growth Factor (NGF) in the cell culture medium. These cells are very well known to develop neurites when they are lead to differentiation using NGF. Glass surfaces were modified by chemisorption of an amino-silane: N[3-(trimethylsilyl)propyl]ethylen-diamine: $C_8H_{22}N_2O_3Si$ (EDA) which contains 3 hydrolysable functions (3MeOH) and lead to grafting by polycondensation. Atomic Force Microscopy (AFM) analysis of substrate topography shows the formation of aggregates and thus the appearing of local gradients in amino-silane surface concentrations. Consequently such local gradients in concentrations induce local gradients in adhesion. Growth of PC12 on such a surface was analyzed by optical microscopy and AFM. Despite the missing of NGF in the cell culture medium, cells were observed to develop a network of axons and neurites on the used modified surfaces. As a negative control, when deposited on a plain surface like poly-L-lysine, cells only showed proliferation behaviour, without developing neurites. These results bring us to the supposition that the cells are able to integrate the physical and chemical cues of the surface when they bind it, thus implicating differentiation and further extension. This study, in which we combine the physical nanostructuration and the local variation in energy of adhesion, may be useful for orienting the axonal outgrowth.

O-635

Photoresponses in H. halobium simulated via systems biology approach

R. Marangoni[1], D. Chiarugi[2], L. Fulgentini[1], G. Colombetti[1]
[1]Istituto di Biofisica del CNR, Pisa, Italy, [2]Dipartimento di Matematica e Informatica, Università di Siena, Italy

Halobacterium halobium is able to react to light by altering its motile behaviour. Because of the presence of different photoreceptor molecules and transduction chains, this bacterium shows complex responses to light stimuli. Red-orange light is sensed through Sensory Rhodopsin I (SRI) and acts as an attractive stimulus that lowers the tumbling frequency. Blue-green light is sensed through Sensory Rhodopsin II (SRII) and represent a repellent stimulus that raises the tumbling frequency. In this work we propose a model of the *H. halobium* photosensorial metabolic pathway that carries out the transduction of the phototactic signal. The model also describes the photocycles of SRI and SRII in response to specific light stimuli. To build up our model we used the π-calculus process algebra, a formal language developed in theoretical computer science, and tailored for the specification of concurrent systems (i.e. a set of entities capable of computing autonomously that cooperate to achieve common goals). Since the paradigm of concurrency can be applied to describe biological systems (and, in particular, biochemical networks) it turns out that the π-calculus is suitable for their description. We implemented our theoretical model in a software tool, that allowed us to perform computer simulations. To describe each elementary biochemical reaction, our tool uses an implementation of the Gillespie's algorithm that allows the simulation runs to happen stochastically. By means of our toolkit we are able to reproduce several aspects of the complex *H. halobium* photobehavior, including adaptation.

Abstracts

– Chemotaxis and signal transduction –

O-636

Signal amplification and noise in bacterial chemotaxis

V. Sourjik
ZMBH, University of Heidelberg, Germany

Amplification and integration of noisy signals is a challenge to most biological networks. We studied these features in chemotaxis on *Escherichia coli,* which is well studies and has a "minimal" signalling pathway, with five types of receptors, a kinase, a response regulator, a phosphatase, and a simple adaptation system that consist of the receptor methyltransferase and methylesterase. Despite its apparent simplicity, the chemotaxis pathway shows extremely high sensitivity, wide dynamic range and is able to integrate multiple stimuli. We used several fluorescence microscopy techniques, including fluorescence resonance energy transfer (FRET), to quantitatively characterize signal processing by the pathway *in vivo*, and showed that chemotactic stimuli are amplified and integrated by the chemoreceptor clusters, which work as large allosteric complexes. We also analyzed effects of common types of perturbations – changes in temperature and a variation in protein levels – on the pathway performance, and demonstrated that such pathway properties as its steady-state output level and sensitivity are highly robust. Our analysis suggests that the pathway has a simplest possible topology (and lowest protein levels) that would allow chemotactic signalling to be sufficiently robust against perturbations, indicating a strong evolutionary optimization. The main component of the robust design – apparently common to most biological signalling networks – is a presence of opposing enzymatic activities, e.g. kinase and phosphatase or methyltransferase and methylesterase, which balance each other in case of a concerted variation in their levels or activities.

O-637

Chemotactic cell movement and its role development

C. J. Weijer
Wellcome Trust Biocentre, University of Dundee, U.K.

Cell movement is a key mechanism during embryonic development of all higher organisms, which requires high spatio-temporal coordination through cell-cell signalling. We study the mechanisms of cell-cell signalling, signal detection and translation into directed movement in two different experimental systems, the social amoebae *Dictyostelium discoideum,* a simple genetically tractable microorganism, showing a relatively simple starvation induced multicellular development, and during gastrulation in the chick embryo, a model system for early amniote development.

In Dictyoselium we study how travelling waves of the chemoattractant cAMP coordinate the chemotactic movement of thousands of cells to result in its typical multicellular morphogenesis. We also investigate how cells detect gradients of cAMP and translate this information into polarised activation of the actin-myosin cytoskeleton to result in force generation and directed movement up a cAMP gradient.

To investigate whether chemotaxis also plays a major role during the early development of higher organisms, we analyse the migration of mesoderm cells, during gastrulation in the chick embryo. We have found that the movement of mesoderm is controlled by a combined action of chemo-attractants belonging to the family of Fibroblast Growth Factors (FGF's). We are now investigating how these factors are detected and transduced to result in directed cell movement and how signalling and movement are integrated to result in proper gastrulation.

Abstracts

– Single molecule fluorescence –

O-638

The influence of cations on the cluster mobility of LFA-1 in living cells

G.-J. Bakker[1], A. Cambi[2], B. Joosten[2], T. Schmidt[3], C. G. Figdor[2], M. F. García-Parajó[1]

[1]BioNanophotonics group, IBEC, Barcelona, Spain, [2]Tumor Immunology Lab., NCMLS, Radboudt Univ. Nijmegen, Netherlands, [3]Dep. of Biophysics, Leiden Univ., Netherlands

The integrin LFA-1 (αLβ2, lymphocyte function-associated antigen-1) is the most important integrin expressed by leukocytes that regulate lymphocyte migration and the initiation of the immune response through binding to ICAM-1, -2, or -3[1]. High-resolution mapping of LFA-1 surface distribution on resting monocytes revealed that LFA-1 organizes in two subpopulations of pre-formed nanoclusters: an unprimed inactive population and a primed, ligand independent, pro-active population[2]. The diffusion profiles of the primed and unprimed LFA-1 and how cluster dynamics, affinity and avidity interplay to regulate monocyte adhesion are yet unknown.
We have applied single molecule epi fluorescence microscopy to study the mobility of the total- and the primed subpopulation of LFA-1 dependent on the presence of cations which are known to modulate the LFA-1 activation state. Both populations show similar dynamical behavior: for both we recover two mobile fractions and a small immobile fraction. The dominant mobile fractions show Brownian motion and do not change significantly in diffusion constant or fraction size upon changes in extracellular Ca^{2+}concentration and/or the presence of Mg^{2+}. Currently, we are also investigating the effect of Mn^{2+} and the role of the cytoskeleton on LFA-1 cluster mobility. [1] Kooyk et al., *Curr. Op. Cell Biol.,* 12:542, 2000 [2] Cambi et al., *MCB,* 17:4270, 2006.

P-639

Single molecule fluorescence studies of Photosystem II-enriched membranes

W. L. Dang, D. R. Klug, L. M. C. Barter

Department of Chemistry, Imperial College, London, UK

Water-splitting, photosynthetic organisms contain the Photosystem II (PSII) reaction centre, which has a manganese-calcium cluster forming its catalytic site. The chemical problem that has been solved by this enzyme is the ability to control the 4-electron reduction of oxygen by creating stable intermediates whilst avoiding high energy losses via relaxations. PS II creates a transmembrane potential gradient by pumping electrons and protons in opposite directions across the thylakoid membrane. The plant has to decouple these reactions not just once, but four times to drive the oxygen evolving complex through five intermediate states, with the release of O_2 occurring only in the last step. It is striking that the 4-electron electrochemistry that oxygen-evolving organisms successfully achieve remains unmatched by chemists, and moreover our understanding of these mechanisms is poor.
Single molecule microscopy has emerged as a powerful tool to examine the heterogeneity among photosynthetic complexes and can be employed to reveal dynamics associated with rate limiting steps or pathways that are hidden in ensemble experiments. Single molecule fluorescence studies using wide-field total internal reflection microscopy have been carried out on PSII-enriched membranes. The observance of particles with Q_A in oxidised or reduced states in individual membranes yields information about the trapping in the reaction centre and allows mechanisms of recombination and the relative efficiencies of different pathways to be revealed. This technique will also allow the catalytic water-splitting reaction in individual reaction centres to be probed.

P-640

A novel approach to probe the activity of single helicases

N. Fili[1], C. Batters[2], M. I. Wallace[3], M. S. Dillingham[4], M. R. Webb[1], J. E. Molloy[1]

[1]MRC NIMR,London,UK, [2]MRC Laboratory of Molecular Biology,Cambridge,UK, [3]Chemical Research Laboratory,University of Oxford,UK, [4]Department of Biochemistry,University of Bristol,UK

DNA helicases are multi-functional motor proteins, which couple translocation along DNA with diverse enzymatic activities. Resolving such complex biochemical properties requires the ability to detect rare and short-lived stochastic events. Unlike bulk measurements, single molecule approaches fulfil this requirement. Here, we present a novel fluorescence-based assay, which allows us to monitor DNA unwinding by single helicases. Biotinylated dsDNA fragments were specifically immobilised on poly-ethylene glycol-coated surfaces through biotin-streptavidin interaction. Total internal reflection fluorescence (TIRF) microscopy was used to achieve high signal-to-noise ratio. The helicase activity was probed by a fluorescently labelled version of the E.Coli single-stranded DNA-binding protein (SSB), which preferentially binds ssDNA. Single, helicase-mediated DNA unwinding events were observed as fluorescent spots of increasing intensity, as increasing numbers of SSB molecules bound to the ssDNA product. Using an objective-based TIRF microscope, these events were recorded at video rate. Increase in the fluorescence intensity directly correlates to the unwinding rate of single helicase molecules. We have used this assay to study the properties of the helicase, AddAB, of B. Subtilis. Recombinant AddAB was allowed to bind the free dsDNA ends and its activity was stimulated by addition of ATP. This assay adds to the single molecule toolbox available for studying DNA processing enzymes.

P-641

The coil-globule transition of barstar

H. Hofmann[1], R. P. Golbik[1], M. Wunderlich[2], C. Huebner[2], R. Ulbrich-Hofmann[1]

[1]Institute of Biochemistry and Biotechnology, Martin-Luther University Halle-Wittenberg, 06120 Halle, Germany, [2]Institute of Physics, Martin-Luther University Halle-Wittenberg, 06120 Halle, Germany

Although it is well-known that unfolded polypeptide chains undergo a collapse upon transfer from denaturing to native conditions, the forces determining the dynamics and the size of the collapsed form have been not yet understood. Here we use single-molecule fluorescence experiments on freely diffusing molecules of the small protein barstar to characterize the unfolded subpopulations under native-like conditions, which is not possible with conventional ensemble measurements. A variant of barstar is labelled by a couple of fluorescent donor and acceptor dyes at the amino acid positions 12 and 89, and the energy transfer efficiency is observed in guanidinium chloride (GdmCl) and urea. A decrease of the concentration of denaturant leads to a gradual compaction of the unfolded protein as indicated by a decreasing end-to-end distance between the two attached dye molecules. No differences are observed when comparing the collapse behaviour above 3.5 M GdmCl or urea. Below this critical concentration, however, the collapse behaviour in GdmCl differs significantly from the behaviour in urea. The predicted end-to-end distances for the unfolded polypeptide chain in water differ by 12 Å when extrapolated from the data taken in GdmCl and urea. This discrepancy might be caused by the ionic nature of GdmCl. This hypothesis is verified by adding KCl to the unfolded barstar at low urea concentrations resulting in a more compact globule.

Abstracts

– Single molecule fluorescence –

P-642

Single molecule fluorescence studies of transcription initiation

L. C. Hwang[1], K. Lymperopoulos[1], S. Ho[2], Y. Wang[2], E. Kortkhonjia[2], S. Weiss[2], A. N. Kapanidis[1]
[1]Dept of Physics and IRC in Bionanotechnology, University of Oxford, Oxford, UK, [2]Dept of Chemistry and Biochemistry and Dept of Physiology, UCLA, Los Angeles, California, USA

Transcription initiation is arguably the most important step in gene regulation. Using single-molecule fluorescence resonance energy transfer (FRET) combined with alternating-laser excitation; we study the mechanism of initial transcription by *Escherichia coli* RNA polymerase (RNAP) associated with initiation factor σ^{70}. This process is also known as abortive initiation, since it involves iterative synthesis and release of short RNA.
Under conditions during which most complexes were active, we observed DNA compaction ("DNA scrunching") during abortive-RNA synthesis. Our results support an initial transcription mechanism during which downstream DNA is "reeled" in and out of the main RNAP channel during each cycle of abortive-RNA synthesis.
We also observed that the width of FRET distribution corresponding to the RNAP-promoter DNA open complex decreases upon addition of the initiating dinucleotide, suggesting that the single-stranded DNA portion surrounding the transcription start site has significant lateral flexibility. This flexibility may allow RNAP to initiate from various sites surrounding the main transcription site. In contrast, during abortive-RNA synthesis, the width of the FRET distribution corresponding to initial transcribing complexes increases dramatically, presumably due to the existence of multiple populations of complexes that do not interconvert during its timescale of diffusion.

O-643

New methods in single molecule fluorescence

D. Klenerman
Department of Chemistry, Cambridge University, U.K.

Single molecule methods have been used reveal new details of biological and cellular processes that are undetectable using ensemble methods. However new methods are needed to study biological systems of increasing complexity and to probe biomolecules under non-equilibrium conditions.
To address these points we have developed two colour coincidence detection to identify macromolecular complexes, in an excess of the individual components, and determine the stoichiometry of these complexes. Our method uses the experimental data alone to determine the optimum threshold for detection of fluorescence bursts and number of chance coincidence events, without the need to run control experiments, hence can deal with the inherent sample-to-sample variability in complex biological preparations or living cells. This method has been used to study the stoichiometry and enzymatic activity of human telomerase and the interaction between proteins on the surface of live T cells. Most single molecule measurements are made under equilibrium conditions so we have developed a simple nanomixer, based on a nanopipette, that allows us to perform non-equilibrium studies in free solution and hence does not degrade the signal to noise. This has been used to study the unfolding of yellow fluorescent protein.

P-644

PicoGreen signalling in DNA condensation by simultaneous FCS and TCSPC

T. Kral[1], N. Adjimatera[2], A. Benda[1], I. S. Blagbrough[2], M. Hof[1]
[1]Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Department of Pharmacy and Pharmacology, University of Bath, Bath, U.K.

We present simultaneous time-correlated single-photon counting (TCSPC) and fluorescence correlation spectroscopy (FCS) study to monitor the behaviour of PicoGreen (PG) on DNA at low-labelling ratio (40 dye/kbp) through its condensation process. Fluorescence correlation spectroscopy (FCS) is a powerful spectroscopic tool to study supramolecular dynamics with single molecule sensitivity and it was proved to be a sensitive method for the characterization of DNA condensation. Measurement and analysis of fluorescence decay kinetic by means of time-correlated single photon counting (TCSPC) is well established technique to get insight into the molecular photophysics. Lipopolyamines (N^4,N^9-dioleoylspermine and N^1-cholesteryl spermine carbamate) were designed and used to condense two plasmid DNAs (pGL3 and pEGFP). Compounds are comprised of tetra-amine spermine which neutralised the DNA phosphate groups and a lipid moiety to facilitate the DNA nanoparticle formation. The aim of the study is to understand more on the behaviour of PG and its fluorescent signalling in DNA condensation. The simultaneous determination of the fluorescence decay kinetics and the FCS read out parameters diffusion coefficient and particle number distinguish between dye release and DNA condensation. Thus, this approach gives much more detailed information on the interaction between lipopolyamines and the PG labelled plasmids. Financial support by the Czech Academy of Sciences (T. Kral via IAA400400621) is gratefully acknowledged.

O-645

Real-time single molecule microscopy in living cell nuclei

U. Kubitscheck, D. Grünwald, J. Ritter, R. Veith, J.-P. Siebrasse
Institute of Physical and Theoretical Chemistry, D-53115 Bonn, Germany

The nuclei of mammalian cells are highly organized structures, in which complex processes such as DNA transcription and replication or RNA processing occur in specific nuclear domains. We develop and apply highly sensitive and fast fluorescence microscopic techniques for the examination of the molecular dynamics in such nuclear domains at the single-molecule level. New insights into the dynamics of the nuclear pore complexes in the nuclear envelope, endogenous mRNA molecules within the nuclei of salivary gland cell nuclei of Chironomus tentans – the so-called Balbiani Ring particles – and non-functional tracer particles and molecules within living cell nuclei will be presented. The given examples demonstrate that the light-microscopic tracking of single protein molecules and ribonucleoprotein particles allows fascinating insights into the intracellular pathways of single protein factors, the structure and dynamics of supramolecular complexes and the elucidation of biomolecular interactions in vivo.

Abstracts

– Single molecule fluorescence –

O-646

The effect of monastrol on the motility of a Eg5-head/DmKHC-stalk chimera

S. Lakämper[1], M. J. Korneev[2], S. Reiter[1], L. C. Kapitein[2], E. Peterman[2], C. F. Schmidt[1]
[1]GAU Göttingen, Germany, [2]VU Amsterdam, The Netherlands

Native Eg5 is a homo-tetrameric motor which is essential for the formation of the mitotic spindle in *Xenopus* laevis egg-extracts. Due to the class-specific formation of anti-parallel coiled-coils of the neck and stalk domains, the extended tetramer exposes two motor domains at each end, reminiscent of conventional kinesin. While it was difficult to test for the processivity and force generation of specifically only one end, native tetramers were observed to produce low forces, possibly indicating regulatory effects mediated by the proximal tail/BimC-domain. Only very recently the problems to obtain a stable dimeric Eg5-motor by mere truncation have been overcome and allowed to show that Eg5-dimers move processively and produce high forces. Here, we present an alternative approach using dimeric Eg5-head/DmKHC-tail chimeras to investigate details of Eg5-motility. The speed of and force produced by single chimeras is similar to native, truncated dimers, but we observe a much higher run length. Interestingly, and in contrast to current models, we observe a sharp exponential decrease in processivity, but not speed by the small Eg5-specific inhibitor, Monastrol. We expect that further chimeric constructs will allow us to gain further insight into the molecular mechanisms important for Eg5-function and regulation within the context of the spindle apparatus.

P-647

Single-molecule fluorescence microscopy in vivo on the twin-arginine translocation (Tat) system

M. C. Leake[1], N. P. Greene[2], R. M. Godun[1], T. Palmer[3], R. M. Berry[1], B. C. Berks[2]
[1]Oxford Physics, Oxford University, [2]Oxford Biochemistry, Oxford University, [3]John Innes Centre, Norwich.

The twin-arginine translocation (Tat) system is responsible for transporting natively folded proteins across prokaryotic cytoplasmic membranes, independent of the Sec mechanism. Using *in vivo* fluorescence microscopy on genetic fusions of yellow fluorescent protein (YFP) to components of the Tat machinery in single, functional *Escherichia coli* cells we show evidence for the diffusion of Tat complexes using single-particle tracking in real-time and provide measurements for the cluster stoichiometry to a precision of single molecules.

O-648

Brightness analysis of isolated mitochondria doped with TMRE and Mitotracker

I. Perevoschikova, Y. Antonenko, D. Zorov
Belozersky Institute and School of Bioengineering and Bioinformatics, Moscow State University, Moscow 119992, Russia

Membrane potential of mitochondria is considered to be essential for cellular function. It is usually estimated using permeable cations or potential-sensitive fluorescent dyes. Here we used a fluorescence correlation spectroscopy (FCS) setup to measure fluorescence intensity trajectories in suspensions of mitochondria, submitochondrial particles and liposomes. The diffusion time, tD, determined from the autocorrelation function G(t), can be related to the mean dwell time of the fluorescent particles within the observation volume, and thereby to their diffusion coefficient. It was found that tD of mitochondria, submitochondrial particles and liposomes was about 210 ms, 100 ms and 13 ms, respectively. Energized and nonenergized mitochondria showed similar values of tD. The addition of succinate in the presence of rotenone increased the brightness of fluorescent particles in the case of mitochondria doped with tetramethylrhodamine ethyl ester (TMRE). By contrast, energization of Mitotracker Red-doped mitochondria under the same conditions led to a decrease in the brightness of the particles, which was reversed by the addition of dinitrophenol. In the case of the negatively charged dye oxonol VI used with submitochondrial particles, the brightness increased after succinate addition and then dropped after the addition of the uncoupler gramicidin A. Therefore, there was a good correlation between the membrane potential and the brightness of the particles under our experimental conditions. Thus, the FCS setup can be used for analysis of energization of mitochondrial populations in suspensions.

P-649

Fluorescence observation of the helicase activity of transcription termination factor Rho

C. Piat[1], A. Schwartz[2], F. Jacquinot[2], A. Rahmouni[2], M. Boudvillain[2], E. Margeat[1]
[1]Centre de Biochimie Structurale, CNRS UMR5048, INSERM U554, and Universités Montpellier I & II, Montpellier France, [2]Centre de Biophysique Moleculaire, CNRS UPR4301, Orleans, France

In *Escherichia Coli*, binding of the hexameric Rho protein to naked C-rich regions of the nascent RNA transcripts initiates Rho-dependant termination of transcription. Rho exhibits *in vitro* RNA-dependant ATPase and helicase activities, but the actual molecular mechanisms used by Rho to disrupt the network of interactions that cement the transcription complex remain elusive. In order to fully characterize these mechanisms, we used in this work a combination of fluorescence spectroscopy approaches to monitor in real time the translocation of Rho on the RNA chain and its helicase activity. Using fluorescence anisotropy, we were able to monitor the directionality and kinetics of Rho activity. Additionally, we used Förster Resonance Energy Transfer (FRET) at the single molecule level to monitor the distance changes between fluorescent probes placed on DNA, RNA, and Rho, in order to fully understand the Rho translocation and helicase processes.

Abstracts

– Single molecule fluorescence –

P-650

Fluorescence brightness and stability dependence of single dyes on the nano-environment

V. Quercioli, C. Bosisio, M. Collini
Dipartimento di Fisica, Università di Milano Bicocca, Milano, Italy

The possibility to detect the fluorescence signal of single molecules opens the way to a variety of studies in the biological and chemical field. To this purpose two main approaches are known: observing single molecules trapped in porous matrices or while flowing through the excitation volume in a diluted solution. We report a study of the effect of the nano-environment (porous matrix or solution) on the photo-stability and the brightness of the molecules by single molecule micro-spectroscopy and fluorescence correlation spectroscopy.

P-651

Rubredoxin: from ensemble to single molecule level

A. M. Santos[1], F. Folgosa[2], A. Fedorov[1], P. Tavares[2], I. Moura[2], J. G. Martinho[1]
[1]Centro de Química-Física Molecular, Universidade Técnica de Lisboa, Portugal, [2]Requimte/Centro de Química Fina e Biotecnologia, Universidade Nova de Lisboa, Portugal

The full understanding of the complex biological systems that support life is fundamental to our modern world. Several biological mechanisms at the molecular level are still barely known, and so its precise physical-chemical characterisation is essential. Rubredoxin from d*esulfovibrio vulgaris* (PDB: 7RXN) is a small electron transfer protein with 52 residues containing an iron-sulphur centre. In order to investigate rubredoxin folding, preliminary photophysical studies were done using its single Trp37 residue as probe. The close proximity of the iron centre (7.7 Å) influences Trp37 spectroscopic properties. For this reason the apo and zinc substituted rubredoxin were also investigated. The protein quantum yields were found to be very low in the case of native form 0.017 and 0.40 for zinc-substituted rubredoxin. The UV-Vis spectrum of the native form exhibits four main bands with maxima at 279, 378, 493 and 570 nm. The first band results from aromatic residues (Tyr, Trp) and the last three bands are due to sulphur-to-iron charge transfer. The emission spectrum exhibit a unique band attributed to Trp37 fluorescence with maximum 331 nm, meaning that its environment is highly hydrophobic. The fluorescence decays of apo and Zn II rubredoxin solutions were obtained at λ_{exc} = 298 nm. The ZnII rubredoxin decays are bi-exponential (1.5, 2 ns) and apo rubredoxin decays are multi-exponential (3, 1.3, 0.35, 0.043 ns). Rubredoxin will be mutated and labeled with a specific dye for future folding studies at the single molecule level.

O-652

Addressing plasma membrane structure at the nanometer length scale

S. Wieser[1], M. Brameshuber[1], M. Moertelmaier[1], E. Fuertbauer[2], H. Stockinger[2], G. J. Schütz[1]
[1]Biophysics Institute, Johannes Kepler University Linz, Altenbergerstr.69, A-4040 Linz, Austria, [2]Dep. of Molec. Immunol., Medical University of Vienna, Lazarettg. 19, A-1090 Vienna, Austria

The plasma membrane structure affects interactions between membrane constituents by influencing their movements at the nanometer scale. We apply single molecule fluorescence microscopy to resolve the plasma membrane structure at a nanoscopic length-scale by employing the high precision for localizing biomolecules of down to 15nm. We applied this technology to study the motion of single glycosylphosphatidylinositol- (GPI-) anchored proteins in the plasma membrane of living cells [1]. In contrast to results obtained by tracking gold-labeled membrane proteins, the single molecule fluorescence data reveal free Brownian motion of the proteins down to length scales of ∼70nm, indicating no constitutive confinement zones. In addition, we developed a technique to detect molecular cluster formation in the cellular plasma membrane of living cells [2]. With this methodology, individual aggregates can be selectively imaged, and the load of each cluster can be determined. Using this technology, we measured the association of a fluorescent lipid analogue in living Jurkat T cells, shedding new light on the current debate concerning the existence of "lipid rafts".

1.Wieser, S., Moertelmaier, M., Fürtbauer, E., Stockinger, H. & Schütz, G. J. Biophys. J., in press (2007).
2.Moertelmaier, M., Brameshuber, M., Linimeier, M., Schütz, G. J. & Stockinger, H. Appl Phys Lett 87, 263903 (2005).

Abstracts

– Electron transfer –

P-653

The reaction of cytochrome bd with oxygen: discovery of the peroxy state

I. Belevich[1], V. B. Borisov[2], N. P. Belevich[1], M. I. Verkhovsky[1]
[1]HBG, Inst. of Biotechnology, University of Helsinki, PB 65, 00014, Helsinki, Finland, [2]Dept. of Molecular Energetics of Microorganisms, Belozersky Inst., Lomonosov Moscow State University, Moscow 119992, Russia

The reaction of oxygen reduction to water catalyzed by respiratory terminal *bd*-type quinol oxidases from *Escherichia coli* and *Azotobacter vinelandii* was followed in real time by multichannel optical spectroscopy and electrometry. The reaction of the fully-reduced enzyme with oxygen proceeds to the final ferryl state through two more intermediates. During the first step oxygen binding to heme *d* yields formation of oxy-complex with the rate constant $1.96\times10^{9}\mathrm{s}^{-1}\mathrm{M}^{-1}$. Surprisingly, the oxygen concentration dependence of the observed rate is different in the different redox-states of the enzyme. In the fully-reduced state the dependence is linear suggesting a simple bimolecular reaction with one-step binding. In contrast, in the one electron-reduced state it is hyperbolic implying a two-step process where binding of oxygen to a transient site precedes its binding to heme *d*.
In the fully reduced enzyme the oxy-complex turns into "true" peroxy intermediate (d^{3+}-O-O-(H)) with the rate constant 126 000 s^{-1} (8 μs). The kinetic spectrum of this transition step shows that the formation of the peroxy state from the oxy-complex goes along with the oxidation of heme b_{595}. The next transition into ferryl state occurs with the rate constant 42 $000\mathrm{s}^{-1}$ (24 μs) and is coupled to the oxidation of the low spin heme b_{558}.

P-654

Characterization of NO-Synthases catalytic intermediates by cryoreduction and Raman and EPR spectroscopies

S. Bernad[1], C. Sicard-Roselli[2], J. Santolini[1]
[1]CEA, iBiTeCs, SB2SM, URA 2096, LSOD, Gif sur Yvette, F-91191, [2]Laboratoire de chimie-physique, CNRS-UMR 8000, université Paris Sud, bât 350, F-91405 Orsay Cedex, France

NO-Synthases (NOS) catalyze two steps of oxidation/hydroxylation that converts L-arginine (Arg) into L-hydroxy-arginine (NOHA) and finally to citrulline and nitrogen monoxide (NO). In the first step the reduction of the native ferric enzyme and the binding of O_2 lead to the build up of $Fe^{II}O_2$ complex. A second electron, provided by a unique cofactor tetrahydrobiopterin (BH_4), allows O_2 activation presumably via the build up of peroxo, hydroperoxo and oxoferryl intermediates. The sequence of electron/protons transfer promoting the O-O heterolytic cleavage is unknown yet and the roles of BH_4 and Arg guanidinium moiety remain unclear.
Our goal is to precise the mechanism of O_2 activation and the factors intervening in the heterolytic cleavage of the O-O bond. We will focus on the synchronous electron/proton transfer and we will especially try to determine the roles of the substrate and of the cofactor BH_4 on the nature of the reaction intermediates by using analogues. We will analyze the first intermediate $Fe^{II}O_2$ species that will be trapped by freeze-quench techniques and characterized at cryogenic temperature by resonance Raman and EPR spectroscopies. We will then use ^{60}Co cryoradiolysis to directly trigger the reaction at cryogenic temperature (77 K). Upon temperature annealing, we will try to determine by Raman and EPR spectroscopies the nature and properties of NOS catalytic intermediates.

O-655

Thermodynamic properties of the redox centers in aa3-type cytochrome c oxidase

D. A. Bloch, N. P. Belevich, M. I. Verkhovsky, M. Wikström
Institute of Biotechnology, P.O. Box 65 (Viikinkaari 1), University of Helsinki, 00014 Helsinki, Finland

The aa_3-type cytochrome *c* oxidase (C*c*O) from *Paracoccus denitrificans* has been characterized using spectropotentiometry. In the *Paracoccus* C*c*O, the titration profiles for hemes *a* and a_3 are both split into two apparent transitions, as for a pair of strongly interacting sites. The low-potential transition (+215 mV *vs. NHE*, pH 8) constitutes to a greater extent of the heme a_3, whereas the high-potential transition (+365 mV) has a larger contribution from heme *a*. This is clearly distinct from the homologous, mitochondrial C*c*O where the heme contributions are equal at both redox transitions. The Cu_A site appears nearly non-interacting (+265 mV). Redox transitions of Cu_B were tracked indirectly by its influence on the MLCT band of oxidized heme a_3, which also showed a near one-electron transition (+385 mV). The latter, however, does not provide information on *all* possible transitions of Cu_B itself. Modeling the titration profiles for all four redox sites suggests that (*i*) a simple model with the interacting hemes only is insufficient and more interactions should be included; (*ii*) more than two spectral forms are required to represent the spectral transitions of the hemes. When taking these into account, modeling shows better fits but does not provide a unique solution: although the upper asymptotic potentials are nearly model-independent (+360 mV, heme *a*; +345 mV, heme a_3; +290 mV, Cu_A; +365 mV, Cu_B, pH 8), the interactions are essentially model-sensitive and could not be reliably assessed from the current data without independent data for the Cu_B site.

O-656

Franck-Condon factors for intra-protein electron transfer reactions from MD simulation

J. Blumberger[1], M. Park[2], K. S. Kim[2], P. L. Dutton[3]
[1]University of Cambridge, Cambridge, U.K., [2]Pohang University, Pohang, Korea, [3]University of Pennsylvania, Philadelphia, U.S.A.

Reorganization free energy and driving force (Franck-Condon factor) are two essential quantities in biological electron transfer (ET) reactions that are often difficult to determine experimentally. In particular reorganization free energy is highly uncertain or unknown for most oxidoreductases. The prospect of calculating the Franck-Condon factor to a useful degree of accuracy would represent a major step forward in understanding the working principles of some of the most important members of this class of enzymes. We have currently devised a computational scheme that allows us to calculate reorganization free energy and driving force for intra-protein ET reactions. First the conformational space of the protein binding the two redox active cofactors is sampled with classical molecular dynamics (MD). Then the ET energy for ET between the two cofactors is calculated at the quantum mechanical/molecular mechanical level of theory for an ensemble of configurations obtained from classical MD. We present results for ET between the heme c cofactor of cytochrome c (cyt c) and a $Ru(bpy)_2(im)$ chromophore docked to a histidine residue at the surface of cyt c. This is one of the very few systems for which experimental data are available allowing us to benchmark our computational scheme. We set out with a comparison of different density functionals in describing structure and ionization potentials of the redox active cofactors, explain the computational scheme and finally discuss the contributions of the cofactors, single amino acids residues and solvent to the Franck-Condon factor for intra-protein ET.

Abstracts

– Electron transfer –

O-657

Enzymes on the edge - voltammetric studies of a multi-heme cytochrome.

J. Butt, M. Cheesman, G. Kemp, S. Marritt, D. Richardson, J. van Wonderen
Centre for Metalloprotein Spectroscopy and Biology, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, U, NR4 7TJ, U.K.

Over one third of enzymes are oxidoreductases. A powerful route to resolve their activities involves replacing one of their natural redox partners with an electrode [1]. Here we illustrate this approach with voltammetric studies of a multi-heme cytochrome that plays key roles in anaerobic respiration and NO detoxification within the enteric pathogen, *Escherichia coli* [2, 3]. Our studies provide quantitative resolution of the enzyme's interactions with nitrite, nitric oxide and sulfite. In addition, our newly developed MOTTLE technology combines magnetic circular dichroism spectroscopy with *in situ* electrodic control over the protein sample to define the redox activities that underpin interactions with these small molecules [4].

1. Angove H, Cole J, Richardson D, Butt J. *J. Biol. Chem.* 2002, 277, 23374
2. Bamford V, Angove H, Seward H, Thomson A, Cole J, Butt J, Hemmings A, Richardson D. *Biochemistry* 2002, 41, 2921
3. Poock S, Leach E, Moir J, Cole J, Richardson D. *J. Biol. Chem.* 2002, 277, 23664
4. Marrit S, van Wonderen J, Cheesman M, Butt J. *Anal. Biochem.* 2006, 359, 79

O-658

Electron tunneling and conformational change in protein redox reactions

B. R. Crane
Department of Chemistry & Chemical Biology, Cornell University, Ithaca, New York, U.S.A.

To probe how proteins couple conformational processes to electron transfer (ET) reactions, we have characterized photochemically-induced ET in single crystals of Zn-porphyrin-substituted cytochrome c peroxidase (ZnCcP) in complex with cytochrome c (Cc), metal-substituted azurins, and flavin-containing photoreceptors. Site-directed mutagenesis, crystal design, cross-linking, and deuterium isotope substitution has been employed to perturb long-range ET across varied protein-protein interfaces. X-ray diffraction studies coupled with rate measurements allow correlation of structure with reactivity. For well-ordered, tightly-packed crystals, rates of inter-protein ET are dominated by tunneling mechanisms that have been well characterized for mediating charge transfer within proteins. However, ET rates in more loosely associating systems, such as the CcP:Cc complexes, are controlled by conformational effects that are highly sensitive to interface structure and solvation. Coupling between redox state and conformational switching is also observed in a fungal light sensor, where modest structural changes accompanying flavin reduction propagate to large scale structural changes in the polypeptide that ultimately send signals within the cell.

P-659

Inside the stability of two species of cytochrome b5

B. Desmolaize[1], G. Truan[2], D. Pompon[2], M. Desmadril[3], E. Guittet[1], C. van Heijenoort[1]
[1]Institut de Chimie des Substances Naturelles (ICSN), CNRS, Gif sur Yvette, France, [2]Centre de Génétique Moléculaire (CGM), CNRS, Gif sur Yvette, France, [3]Institut de Biochimie et Biophysique Moléculaire et Cellulaire (IBBMC), CNRS, Orsay, France

Cytochrome b5 is a membrane-bound protein. As other cytochrome it plays an essential role in many physiological pathways where it is involved as an electron carrier. We focused particularly on electron transfer between two types of cytochrome b5 (human and yeast) and one kind of cytochrome P450 A (CYPA). In a rebuilt human system containing the human cytochrome b5 and CYPA an activity has been observed and never when the human cytochrome b5 is replaced by the yeast form. In order to understand the difference in activity for CYPA between human and yeast cytochrome b5 and to eventually relate them to particular fragments of the proteins, we studied the structure of both species by NMR. The first results provided differences in the behavior of the two species. The equilibrium is very long to obtain for the yeast form, phenomenon not observed in the case of the human form. This observation brought us to study the stability of the two proteins. The stability was investigated by thermal and chemical denaturation experiments and we determined the affinity of the two cytochromes b5 for heme group. The results obtained by various methods (NMR, microcalorimetry, circular dichroism, and UV/visible spectroscopy) don't present significant difference thermodynamically. Nevertheless the affinity of cytochrome b5 for heme is slightly higher for the human than for the yeast form and may explain the difference observed in the behavior of the two species.

P-660

Polarized transient absorption to distinguish tryptophans in the photolyase electron transfer chain

A. Espagne[1], S. Villette[1], A. Eker[2], K. Brettel[1], M. Byrdin[1]
[1]CEA, iBiTecS, Gif-sur-Yvette, France, [2]Medical Genetics Center, Erasmus University, Rotterdam, The Netherlands

Transient absorption spectroscopy is a powerful tool to study biological electron transfer chains, provided that the members of the chain give rise to distinct changes of their absorption spectra. There are, however, cases where part of the chain is made of identical molecules so that electron transfer between them does not change absorption. A prominent example is the chain FADH°-W382-W359-W306 in DNA photolyase from *E. coli*. Upon absorption of a photon, the excited state of FADH° abstracts an electron from the tryptophan residue W382 in ~30 ps (monitored by transient absorption). The cation radical $W382^{\circ+}$ is presumably reduced by W359, and $W359^{\circ+}$ is finally reduced by W306. The latter two reactions could not be monitored by transient absorption as the absorption changes due to reduction of $W382^{\circ+}$ ($W359^{\circ+}$) are compensated by those due to the oxidation of W359 (W306). To overcome this difficulty, we use the fact that polarized excitation induces a preferential axis (that of the excited flavin transition) in the system, and that W359 and W306 form different angles with that axis (known from the crystal structure). Thus, polarized detection should allow distinguishing between them. To demonstrate this, we replaced W306 with redox inert phenylalanine, thus pruning the chain behind W359. We show that the resulting transient absorption polarization pattern on a nanosecond time scale is in line with the orientation of W359, and well different from that in wild type photolyase where W306 is finally oxidized. This paves the way towards a transient detection of inter-tryptophan electron transfer.

Abstracts

– Electron transfer –

P-661

The mutant I(L177)H reaction center of Rhodobacter sphaeroides: properties and pigment composition.

T. Y. Fufina, L. G. Vasilieva, R. A. Khatypov, I. I. Proskuryakov, A. A. Zabelin, A. Y. Shkuropatov, V. A. Shuvalov
Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, 142290, Russia

In *Rb.sphaeroides* the conversion of light energy takes place in photosynthetic complex termed reaction center (RC). The RC consists of three protein subunits and 10 cofactors of electron transfer. We have studied how the substitution of Ile to His in the position L177 affects the properties of RC. *Rb. sphaeroides* I(L177)H mutant forms stable photochemical active RC complexes, and the quantum yield of charge separation in the mutant RC was decreased. Spectral and photochemical properties of the mutant RC differ significantly in the absorption bands corresponding to BChl molecules. It was shown that the pigment extract from the mutant RC contains only three BChl molecules, comparing to four BChl molecules in the pigment extract from the wild type RC. Initially it was suggested that a BChl molecule might be missing from the mutant RC, but additional data (LT absorbance spectra, FTIR spectra, Em P/P+) were inconsistent with this proposal. It was shown that after pigment extraction by different organic solvents one BChl molecule per the mutant RC remains tightly bounded to the protein. Using SDS PAGE we have demonstrated that in the sample of I(L177)H RC a green pigment line moves through the gel together with the L-subunit. We have made a conclusion that in the mutant RC I(L177)H substitution caused covalent attachment of a BChl molecule to L-subunit.
Authors acknowledge the support by RFBR (06-04-48686, 05-04-49129) and RAS.

P-662

Exploring redox-dependent proton transfer in cytochrome c oxidase by ATR-FTIR

E. Gorbikova, M. Wikström, M. I. Verkhovsky
Helsinki Bioenergetics Group, University of Helsinki, Finland

Cytochrome *c* oxidase (C*c*O) is a terminal complex of respiratory chain creating electrochemical proton transmembrane gradient. The enzyme includes a 'wire' of redox centers responsible for electron-transfer (ET) from cytochrome *c*. 8 electrons are transferred to the reaction center where they take part in oxygen reduction till water. Energy of ET is used to translocate 4 protons across the membrane (in aa_3 type) and take up 4 more protons for oxygen reduction. One useful technique that can shed light into redox-dependent proton transfer in C*c*O is ATR-FTIRs (Attenuated Total Reflectance Fourier Transform InfraRed spectroscopy). Here, potential to detect protonation status of residue is joined with possibility to change buffers along measurement. Applying electrochemical titration by ATR-FTIRs, we defined redox behavior of each of four redox centers from where concluded that C*c*O takes up 2 protons for oxygen chemistry on the enzyme reduction: one associated with Cu_B center reduction and one – with both hemes reduction (1). pH-Dependent redox changes in FTIR mode showed uptake of nonspecific diffuse proton besides two protons for chemistry. One redox-active water molecule was identified by the same approach. The feature found belongs to water molecule that is produced in the reaction center or to some other water molecule that loses H-bond upon reduction (2).
1. Gorbikova, E. A., Vuorilehto, K., Wikström, M., and Verkhovsky, M. I. (2006), *Biochemistry 45*, 5641-5649.
2. Gorbikova, E.A., Belevich N.P., Wikström, M., and Verkhovsky, M. I. (2007), *Biochemistry 46,* 4177-4183

O-663

Electrodes for redox-active membrane proteins

L. J. C. Jeuken[1], R. J. Bushby[2], S. D. Connell[1], N. N. Daskalakis[1], A. Erbe[1], S. D. Evans[1], S. A. Weiss[1]
[1]School of Physics and Astronomy, University of Leeds, Leeds, U.K., [2]Centre for Self-Organising Molecular Systems (SOMS), University of Leeds, Leeds, U.K.

Redox proteins perform a myriad of functions in biology and dynamic electrochemical techniques have proven to be powerful tools to study them. Many redox proteins involved in photosynthesis and metabolism are located in the membrane and the main challenge is to design electrode surfaces that adsorb membrane proteins in their native state on the electrode while efficiently exchanging electrons. Here, we report on three different electrochemical approaches we have developed to study redox-active membrane proteins. In the first approach, cholesterol modified gold surfaces are modified with a planar lipid bilayer by self-assembly of reconstituted proteoliposomes containing the terminal heam-copper oxidase, cytochrome bo_3, from *E. coli*. Using quinone as native mediator it is shown that in this configuration cytochrome bo_3 retains its activity. In the other approaches, total bacterial membrane extracts from *B. subtilis* and *E. coli* are tethered onto gold surfaces either in the form of vesicles or planar bilayers. These total membrane extracts, which still contain all the bacterial membrane proteins and co-factors, remain intact on the surface while the native quinone-pool can be oxidised and reduced electrochemically. The membrane proteins retain their function as is probed by the catalytic activity of a succinate dehydrogenase or cytochrome bo_3, which interact with the quinone pool. These electrode systems can easily be adapted for other organisms' membranes and be used to study their redox-active membrane proteins that interact with the quinone pool.

P-664

Dynamic protonation state analysis of Dsb proteins

D. Narzi[1], C. Stirnimann[2], M. G. Grütter[2], J. Grimshaw[3], R. Glockshuber[3], G. Capitani[2], R. A. Böckmann[1]
[1]Theoretical & Computational Membrane Biology, Center for Bioinformatics, Saarland University, Germany, [2]Department of Biochemistry, University of Zurich, Switzerland, [3]Institute of Molecular Biology & Biophysics, ETH Zürich, Switzerland

Disulfide bond formation and rearrangement is of central importance for the correct folding and stability of several proteins. In prokaryotes this process is catalyzed by Dsb proteins. The highly oxidizing protein DsbA introduces disulfide bonds in the substrate by reduction of its cysteine pair placed in the active site. Subsequently it is reoxidized by the inner membrane protein DsbB, which transfers electrons to ubiquinone. Rearrangement of wrong disulfide bonds is achieved by the protein DsbC, which catalyzes the isomerization of disulfide bonds of misfolded proteins [1,2].
Here we investigated by molecular dynamics (MD) simulations and protonation state analysis the functional properties of various Dsb proteins]. The pKa analysis involved the background interaction energy, the pair wise interaction energy between the titratable groups, as well as the desolvation energy. The inclusion of hydration entropy was found to be important for the correct description of the protonation states of the active site cysteines. For DsbL, we show that tiny structural changes may induce proton transfer from the active site cysteine pair to specific buried residues which we suggest to act as a proton source or sink crucial for the function of the Dsb proteins.
[1] C. U.Stirnimann *et al.* Cell.Mol.Life Sci. 63:1642-1648 (2006)
[2] C.U.Stirnimann *et al.* J.Mol.Biol. 358, 829-845 (2006)

Abstracts

– Electron transfer –

P-665

Regulation of energy transfer in the bacterial FOF1-ATPase by redox potential

A. Poladyan, G. Kirakosyan, A. Vassilian, A. Trchounian
Yerevan State University, 0025 Yerevan, Armenia

The oxidation-reduction states of SH-groups in the bacterial H^+-F_OF_1-ATPase determined by redox potential can modulate the activity of F_OF_1 participating in energy transfer via a dithiol-disulfide interchange between the ATPase and the other proteins [1]. In *E. coli*, the number of SH-groups in vesicles from fermenting cells at pH 7.5 is increased by ATP or by formate suggesting an interaction between F_OF_1, K^+-uptake system and hydrogenase 4 (Hyd-4) or 3 (Hyd-3) [1]. The increase in the number of SH-groups by ATP, formate or Cu^{2+} is absent, the inhibition in ATP-dependent increasing SH-groups number by Cu^{2+} is lacked with *N*-ethylmaleimide (NEM), *N,N'*-dicyclohexylcarbodiimide (DCCD) or sodium azide. The increased level is observed in *hycE* or *hyfR* mutants whereas the ATP-dependent increase is in *hycE* but not in *hyfR* mutants. Both changes disappear in the *atp* or *hyc* mutants deleted for F_OF_1 or Hyd-3, correspondingly. The number of SH-groups in vesicles from *E. hirae* at pH 8.0 is increased by ATP or by NAD^++NADH and inhibited with NEM. The increase is more when ATP and NAD^++NADH both are added. This is lacked by DCCD or sodium azide and also absent in *atp* mutant with defect in F_OF_1 and less in K^+-free medium. The results are correlated with data about K^+-dependent ATPase activity suggesting a relationship between F_OF_1 and K^+ uptake system via a dithiol-disulfide interchange [1] which could be regulated by redox potential. Conformational change in F_OF_1 is suggested leading to modulation of its activity.

[1] A.Trchounian. *Biochem. Biophys. Res. Commun*. 315 (2004) 1051-1057.

P-666

The effect of visible irradiation on the oxygen consumption rate by cultured cells of different taxa

A. Remedi[1], L. Di Salvo[1], S. Lucia[1], G. Cercignani[2], G. Colombetti[1]
[1]Istituto di Biofisica CNR, Pisa, Italy, [2]Università di Pisa, Italy

In the last few years several authors have reported the fast cell recoveries in some kind of pathologies by the irradiation with monochromatic red or near-infrared light. Along the same research theme, the NASA developed some LED's prototypes. The hypothesis has been supported that these effects are mediated by the mitochondrial enzyme cytochrome *c* oxidase (CcOX). We measured, through a Clark electrode, the effects *during* activity assay of visible-to-near-infrared (Vis-NIR) irradiation on the *in vitro* activity of the bovine purified CcOX and on the *in vivo* activity of the same enzyme in cultured cells. We get consistent results showing that the activity of purified CcOX is unaffected by irradiation with any frequency of Vis-NIR light. The same kind of experiments have been carried out on cultured mammalian cells, on *Tetrahymena thermophila* and on *Saccharomyces cerevisiae*. The irradiation with NIR light had no effect on oxygen consumption of the cells; the Vis light, indeed, induced a sudden increase. Similar results are obtained on HaCaT cells and on aerobically grown yeast cells. The dose-response curves made on *T. thermophila* show that most of this effect was due to the blue component (420±30 nm), while red light (> 600 nm) produced a lower effect than blue light according to the absorption spectrum of CcOX. It is interesting that the Vis irradiation on anaerobically grown yeast cells has no relevant effect. These results seem to confirm that CcOX is the specific photoacceptor involved in these phenomena and suggest an underlying mechanism of photomodulation of the respiratory rate in eukaryotic cells.

O-667

Isotope effects and tunneling in enzyme systems

N. S. Scrutton
Manchester Interdisciplinary Biocentre, University of Manchester, UK

We have used combined pressure, temperature and mutagenesis studies to study primary and a-secondary isotope effects to analyse the hydride transfer reaction from NADH to FMN in bacterial morphinone reductase. The reaction is observed directly in a rapid mixing stopped-flow instrument and is kinetically resolved from steps involving coenzyme binding and formation of an enzyme-NADH charge-transfer (CT) complex. In the reductive half-reaction, the 1° KIE for hydride transfer is temperature-dependent and the 2° KIE is exalted, consistent with a need for preorganization in an environmentally coupled H-tunnelling model. The enzyme requires a promoting motion to move the nicotinamide C4-H sufficiently close to the FMN N5 atom to facilitate tunneling, a notion that is consistent with combined pressure and temperature effects on the 1° KIE and numerical modelling of the reaction parameters in the context of an environmentally coupled framework for H-tunneling. We show that mutagenesis induces multiple reactive conformations in the enzyme-substrate complex which are kinetically resolved in stopped-flow studies. Our studies highlight a potential flaw in using steady-state approaches alone in the analysis of tunneling regimes in enzyme systems.

References

[1] Hay, S., Sutcliffe, M. J. & Scrutton, N. S. (2007). *Proc. Natl. Acad. Sci. USA* 104, 507-512
[2] Pudney, C. R., Hay, S., Sutcliffe, M. J., & Scrutton, N. (2006). S. *J. Am. Chem. Soc.* 128, 14053-14058

P-668

Calculation of redox properties: understanding short and long range effects in rubredoxin

M. Sulpizi[1], S. Raugei[2], J. Vandevondele[3], P. Carloni[2], M. Sprik[1]
[1]Department of Chemistry, University of Cambridge, UK, [2]SISSA, Trieste, Italy, [3]Institute of Physical Chemistry, University of Zurich, Switzerland

We show that for rubredoxin [1], a small iron-sulfur protein, it is possible to combine a full *ab initio* description of the electronic structure of the protein in explicit solvent with sampling of the relevant time scale of the protein dynamics using molecular dynamics. Within the framework of Marcus theory [2] we are able to reproduce the experimental redox potential difference of 60 mV between a mesophilic and thermophilic rubredoxin within an accuracy of 20 mV and explain it in terms of contributions from a few residues close to the metal centre. We also compute the reorganization free energy for oxidation of the protein obtaining 720 meV for the mesophilic and 590 meV for thermophilic variant. Decomposition of the reorganization energy shows that this is largely determined by the solvent, with both short range (an oxidation induced change of coordination number) and long range (dielectric) contributions. The 130 meV higher value for the mesophilic form is analyzed in terms of detailed differences in the solvent structure around the metal center and the dielectric response. These results underline the importance of a molecular description of the solvent and of a correct inclusion of the polarization effects [3].

[1] Meyer J, Moulis J-M (2001) Handbook of Metalloproteins (Wiley) pp 505.
[2] Marcus RA, Sutin N (1985) Biochim. Biophys. Acta 811: 265.
[3] Sulpizi M, Raugei S, VandeVondele J, Carloni P, and Sprik M (2007) JPC B 111(15); 3969.

Abstracts

– Electron transfer –

P-669

Synthesis, Characterization, and Excited state properties of New Lanthanum(III) complexes with Amino acid based Azo dyes.

S. Sushma, M. Singh
Delhi University, India

Luminescent lanthanide complexes have long been used as electroluminescence, fluoroimmunoassay and laser materials because of their line like and strongly luminescent f-f transitions. Moreover, the emitted light of the lanthanide complexes does not depend on the size of complex but only on the nature of the rare earth ions and the ligands. Transition between f-f orbital of lanthanides are strictly forbidden. Moreover, many f-f transitions are also spin forbidden although spin-orbit coupling attenuates the forbiddeness. Nevertheless, both restrictions have importance. The band have low absorption coefficient and the radiative lifetime of f-f state are rather large. Owing to small absorption coefficient of lanthanides, the excitation can be facilitated by suitable dye which not only act as a chelating ligand, but also absorb the light and subsequently transfer the excitation energy to the emissive metal ion. In this context, we have synthesized lanthanum(III) complexes with aminoacid based, azo dyes by reaction of lanthanum(III) salt and the ligands(dye), in 1:1 ratio. The lanthanum(III) complexes were characterized by elemental analysis, IR, H NMR and UV spectroscopies. The complexes can be efficiently excited with visible light and show intense lanthanides luminescence at low concentrations.

O-670

Transient protein electron transfer complexes studied by NMR

M. Ubbink
Leiden University, The Netherlands

Protein:protein interactions are the basis of many biological processes. Stable protein complexes can be characterized structurally by the same methods as single proteins, but in the case of weak complexes, other methods are required. We have studied a range of weak electron transfer (ET) protein complexes using nuclear magnetic resonance (NMR). By determining the nature of the binding sites it is possible to put forward general properties of interfaces in ET complexes. It has been found that these complexes exist in an equilibrium between a well-defined state and a dynamic, encounter-like state. In some cases, the dynamic state can even be dominant. By taking advantage of paramagnetic effects in NMR, it is also possible to determine the structure of weak protein complexes, even if they are large. This will be illustrated with several examples, the complexes of cytochrome *f* and plastocyanin (39 kDa), cytochrome *c* peroxidase and cytochrome *c* (46 kDa) and nitrite reductase and pseudoazurin (152 kDa). Paramagnetic NMR can also be used to map the degree of mobility in the encounter state, even if it represents only a minor fraction of the complex [1]. In this way, a much more complete picture is obtained of how transient ET protein complexes are formed and what they really look like.

[1] Volkov AN, Worrall, JAR, Holtzmann E, Ubbink M (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103**, 18945-18950.

P-671

Development of a supported cell membrane for investigating redox enzymes

S. A. Weiss, L. J. C. Jeuken
School of Physics and Astronomy, University of Leeds, U.K.

We present the development and characterisation of a supported cell membrane model for the study of integral membrane proteins on electrode surfaces. The advantage of the presented system is that the functionalised surface is formed using whole bacterial cell membranes which contain all membrane proteins and lipids of the native membrane. The planar membrane architecture is formed by self-assembly of proteoliposomes which are made by mixing the cell membranes with lipid extracts to reduce to the lipid-protein ratio. The supported cell membrane model has been characterised using a variety of techniques including tapping mode atomic force microscopy (TM-AFM), electrochemical impedance spectroscopy (EIS), surface plasmon resonance (SPR) and quartz crystal microbalance with dissipation (QCM-D). The functionalised electrode was able to monitor the activity of an ubiquinol oxidase expressed in *E. coli*, cytochrome bo_3 (cbo_3) by cyclic voltammetry. Interfacial electron transfer to cbo_3 is mediated by the membrane-localized ubiquinol, the physiological electron donor of cbo_3. This cell membrane model has allowed us to determine kinetic parameters such as a K_M for oxygen and ubiquinol substrates and inhibition of activity by zinc ions for cbo_3 in a near native environment.

Abstracts

– Ion channels & cancer –

O-672

Unconventional ion channel signalling in cancer cells: interaction with adhesion receptors.

A. Arcangeli
University of Florence, Italy

Recent evidence indicates that the expression and activity of different types of ion channels, especially K^+ channels, mark and regulate specific stages of cancer progression. In some cases, the role of ion channels in these phenomena can be traced back to a complex interaction of ion channel proteins with adhesion receptors, mainly integrins. Integrins can regulate ion channels and form macromolecular complexes, thus contributing to the localization of the channel onto the plasma membrane. The integrin/channel complex regulates downstream signaling proteins, like tyrosine kinases and GTPases. This process may occur in plasma membrane microdomains, such as *caveolae*. It appears that ion channels sometimes transmit their signals through conformational coupling, instead of change in ion fluxes. In addition, the channel protein is not merely a final target, since it often feeds back by controlling integrin activation and/or expression.

We present here evidence for a non conventional signalling mechanism involving K^+ channels belonging to the hERG1 family and β_1 integrins. In particular, a functionally significant complex is formed between the VEGF receptor 1 (FLT-1), the hERG1B protein and β_1 integrins in acute myeloid leukemias (AML). Such a complex regulates VEGF secretion and migration of AML cells. Such a phenotype was also observed *in vivo*. *Herg1* positive blasts were more efficient in invading the peripheral circulation and the extramedullary sites after engraftment into immunodeficient mice. Moreover, *herg1* expression in leukemia patients correlated with a higher probability of relapse and shorter survival periods.

P-673

Erg1 regulates cell proliferation and apoptosis during normal vertebrate limb development

A. C. Certal[1], M. R. Santos[1], J. Rodríguez-Léon[2]
[1]Centre for Developmental Biology, Instituto Gulbenkian de Ciência, 2780-901 Oeiras, Portugal, [2]Center of Regenerative Medicine of Barcelona, E-08003 Barcelona, Spain

Ion dynamics may underlie important mechanisms during vertebrate limb development. ERG K+ channels are key molecular components of the cardiac repolarizing current in humans, although other functions have been suggested. We show that, in chicken embryos, *erg1* is expressed in the limb mesoderm before limb induction until late stages of limb outgrowth. *Erg1* expression is first detected at the prospective forelimb at the same time as *tbx5*, the first known genetic marker for limbs, suggesting a role in limb initiation. Later, *erg1* is involved in limb outgrowth, with transcripts detected in the progression zone and in both necrotic zones. It is also expressed during digit patterning, at the interdigital level and in the phalanxes. Downregulation of *erg1* showed a functional role for this channel. When inhibition of erg1 is performed in the presumptive limb field, the resulting limbs are either truncated or smaller than untreated limbs, suggesting a role for this channel in cell proliferation, as also shown for cancer cells. When this is done in the interdigital tissue of 5 day-old embryos, the autopods remain with interdigital membranes and beads soaked in Erg toxin mimics this phenotype, indicating another role for *erg1* in the regulation of apoptosis. In conclusion, we show that ERG1 has an important function in development which is pivotal for its deregulation in cancer cells.

We thank FCT POCTI/BCI/47972/2002, BPD/14697/2003 and EU Network "Cells into Organs".

O-674

Eag1 potassium channel non-canonical function induces upregulation of cancer genes.

B. R. Downie[1], A. Sánchez[1], C. Contreras-Jurado[1], H. Knötgen[2], M. Gymnopoulos[2], C. Weber[1], W. Stühmer[1], L. A. Pardo[1]
[1]Max-Planck Institute of Experimental Medicine, Göttingen, Germany, [2]iOnGen AG, Göttingen, Germany

Ether-á-go-go-1 (Eag1) is a CNS-localized voltage-gated potassium channel that is found ectopically expressed in most extra-cranial solid tumors. While evidence suggesting an implication of Eag1 in tumor biology has been reported, the mechanisms by which the channel contributes to tumor progression remain elusive. We have used in vivo and in vitro techniques to show that blockers of Eag1 are able to inhibit tumor progression, while eliminating ion permeation by mutagenesis does not totally abolish the oncogenic properties of Eag1. We propose a candidate mechanism in which Eag1 promotes tumor progression independently of ion permeability by non-canonically interacting with key cellular components.

O-675

Electrophysiological effects of estrogen on voltage-gated Na^+ channels in human breast cancer cells

S. P. Fraser[1], I. Ozerlat[1], J. K. Diss[2], M. B. Djamgoz[1]
[1]Division of Cell and Molecular Biology, Imperial College London, London, United Kingdom, [2]Medical Molecular Biology Unit, Institute of Child Health, London, United Kingdom

Estrogen is known to be involved in development and progression of human breast cancer (BCa). We have shown (i) that a voltage-gated sodium channel (VGSC), Nav1.5, in its newly identified neonatal splice form is selectively upregulated in both the human metastatic BCa cell line MDA-MB-231 and primary tumour biopsy tissues and (ii) that VGSC activity enhances a range of metastatic cell behaviours (Fraser et al., 2005). Thus, estrogen signalling and VGSC expression/activity could be functionally associated. Estrogen classically works as a transcriptional regulator but has increasingly been shown to have fast-acting non-genomic effects. We have used whole-cell patch-clamp recording to investigate whether estrogen receptor (ER) signalling could have short-term effects on VGSC activity in MDA-MB-231 cells. Acute application of estradiol (E2) increased VGSC current amplitude in a dose-dependent manner: 10 nM E2 increased current amplitude by $39 \pm 5\%$. Blocking G-protein signalling with GDP-β-S suppressed the effect of E2, whilst GTP-γ-S increased basal VGSC current amplitude. These results are consistent with E2 enhancing VGSC current amplitude via a mechanism involving a G-protein, possibly GPR30, a G-protein coupled ER on the plasma membrane, with PKA or PKC as downstream intracellular secondary messengers.

Reference Fraser SP et al. (2005). Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. *Clin Cancer Res* **11**, 5381-5389.

Abstracts

– Ion channels & cancer –

O-676

On the first passage time distributions for K channels of the cancer cells.

Z. J. Grzywna[1], K. Malysiak[1], M. Rubi[2]

[1]Dep. of Phys. Chem. and Techn. of Polymers, Section of Phys. and App. Math., Strzody 9, 44-100 Gliwice, Poland, [2]Departament De Fisica Fonamental Facultat de Fisica, Marti i Franques, 1 E-08028 Barcelona, Spain

The "ball and chain" model of rapid inactivation for a voltage—gated K^+ channel has been confirmed experimentally in a very solid way. The main idea reads that when the channel is open the wandering "ball" (19 amino-acid terminal) can occlude (inactivate) the channel being linked to its proper by a segment of unfolded polypeptide "chain". If the region of a ball activity is altered, the kinetics of channel inactivation may be altered as well.
Our hypothesis takes into account the dramatic difference between normal and cancer cells K^+ channels behavior. We derived the first passage time distribution for classical and fractional (anomalous) diffusion of a "ball and chain" model. Based on that we predicted the character of "patch" and voltage clamps outputs for healthy and cancer cells.

P-677

Metabolism and transport of racemers in cells distinguished in NMR spectra with chiral tunable-alignment media

P. W. Kuchel, C. Naumann, D. Szekely, W. A. Bubb
School of MMB, University of Sydney, NSW, Australia

Almost-exclusive use of L-amino acids in ribosomal protein synthesis is a prominent example of the 'chiral bias' of living systems; the reason for this is the 'three-point attachment' phenomenon in enzymes. A subtle situation involving chirality exists in human erythrocytes where L-lactate is produced via glycolysis using NAD/NADH, and D-lactate is produced via the glyoxalase pathway using glutathione.
NMR spectra of racemic mixtures of solutes can be resolved if they are constituted in chiral media, and more structural information is obtained if the medium is aligned as well. Gelatin, which is chiral, can be set inside a silicone-rubber tube [*1*] and variably stretched and thus elicit, in NMR spectra, a range of residual dipolar splittings in spin = $\frac{1}{2}$ nuclides, and residual quadrupolar splittings in spin > $\frac{1}{2}$ nuclides. Erythrocytes set and stretched in the device can be studied with respect to transmembrane exchange and metabolism of chiral solutes. D- and L-lactate give clearly resolved spectra so the simultaneous measurement of parallel fluxes in glycolysis and the glyoxalase pathway has been possible for the first time.

Reference

[1] Kuchel, P.W., Chapman B.E., Müller N , Bubb W.A., Philp D. J. and Torres A.M. (2006) *J. Magn. Reson.* **180**, 256-265.

P-678

ABCG2 and the role of nucleotide binding in drug translocation

C. A. McDevitt, G. Hobbs, R. Callaghan
Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, University of Oxford, Oxford, UK, OX3 9DU

Multidrug resistance of cancer is a clinical phenomenon associated with the active efflux of chemotherapeutic drugs from cancer cells. This resistance is mediated by ABC transporters such as P-glycoprotein (ABCB1), the multidrug resistance associated protein (MRP1, ABCC1) and the breast cancer resistance protein (BCRP, MXR, ABCG2). Critical to the translocation of drugs, by ABC transporters, is a switch in the affinity for drug allocrites at the binding sites in the transmembrane pocket. The switch from a high to a low affinity state facilitates drug efflux and occurs as a result of conformational changes transmitted from the nucleotide binding domains. This investigation sought to elucidate whether the switch was triggered by nucleotide binding or the subsequent hydrolytic event. Plasma membranes from insect cells expressing $ABCG2^{R482G}$ isoform were used to characterise the binding of [^{3}H]daunomycin to the multidrug transporter. The effect of nucleotide binding and hydrolysis was probed by modulating ABCG2 affinity for [^{3}H]daunomycin by nucleotide trapping and analogues. The non-hydrolysable nucleotide analogues ATP-γ-S and TNP-ATP, mimicking the nucleotide bound state, produced a reduction in [^{3}H]daunomycin affinity. This low affinity binding was maintained in the post-hydrolytic vanadate trapped state. The data supports the hypothesis that nucleotide binding event *per se* is responsible for the change in the binding site conformation to a lower affinity; thereby supporting the nucleotide switch model proposed for P-glycoprotein.

P-679

Extracellular acidosis modulates electrophysiological activity of 'neonatal' Nav1.5 α-subunit

R. Onkal, M. B. Djamgoz
Division of Cell and Molecular Biology, Imperial College London,U.K.

Protons have been shown to be potent physiological modifiers of voltage-gated sodium channels (VGSCs), affecting the voltage range of channel gating and suppressing inward Na^+ current (I_{Na}) with a pK_a of ∼6. $Na_v1.5$ is the main VGSC expressed in heart muscle and there is evidence that cardiac ion channels may revert to embryonic forms following infraction when ischemic acidosis would be expected to occur. However, how extracellular pH (pH_o) changes would affect 'neonatal' $Na_v1.5$ channel activity has not been investigated. We used whole-cell patch-clamp technique to study the electrophysiological properties of the 'neonatal' $Na_v1.5$ α-subunit, stably expressed in EBNA cells; pH_o of the bathing mammalian physiological saline solution was varied to cover the pathophysiological range (pH_o 5.75-8.25). Acidification of pH_o produced a voltage-dependent decrease in peak I_{Na} amplitude (with a pK_a of ∼6.05). Reducing pH_o from 7.25 (control) to 6.25 caused (1) depolarizing shifts in overall voltage dependence of steady-state activation (∼9 mV) and inactivation (∼5 mV); (2) depolarizing shifts in threshold of activation (from -59 to -53 mV) and voltage for peak (from -19 to -8.5 mV); (3) a progressive slowing and increased voltage-dependence of time to peak; and (4) a complex slowing of inactivation and recovery from inactivation. These effects were rapid and fully reversible. It is possible these pH_o effects relate to the changes in electrophysiological and biochemical properties that occur during cardiac development and acute ischemia of myocytes, as well as during breast cancer metastasis where significant upregulation of 'neonatal' $Na_v1.5$ splice variant has been shown to occur.

Abstracts

– Ion channels & cancer –

P-680

Microrheological measurements inside starfish oocytes

L. Selvaggi[1], G. Pesce[1], A. Sasso[1], L. Santella[2], G. Rusciano[1]

[1]Università di Napoli Federico II, Napoli, Italy, [2]Stazione zoologica nazionale Anthon Dohrn, Napoli, Italy

The mechanical properties of cells are essential for fundamental biological functions such as the alteration of cell shape in response to physiological and mechanical stimulations. These cell responses are related to the cytoskeleton scaffold including actin filaments and other sub-cellular structures.

To investigate cytoskeleton mechanics we study viscoelastic response of living cells by laser-particle-tracking (LPT) technique spanning up to 5-decades of frequency. LPT is fast and sensitive enough to follow mechanical properties variations inside the cells. The A.Auranciacus starfish oocytes are an ideal model for laser-tracking microrheology because they have both a spherical shape and a wide transparency. We explore the viscoelastic properties of starfish oocytes cytoplasm at different stages of maturation by monitoring the Brownian motion of micro-particles (1-μm-diameter polystyrene beads) injected inside the cell. The results show that the viscoelastic modulus amplitude of an immature oocyte is about two orders of magnitude greater than that of a mature oocyte challenged for 1 hour with the maturing hormone 1-metyladenine (1-MA). The decrease in the cytoskeleton rigidity during the maturation process is correlated with the dynamic changes in F-actin networks. Therefore, the cytoskeleton-mediated changes in the viscoelastic properties of the cytoplasm may also play an important role in the intracellular calcium release modulation.

P-681

Cation permeability induced by polyene antibiotics in the membrane of skeletal muscle fibre

N. Shvinka

Latvia Univ. - Inst. of Exp. and Clinical Medicine, Riga, Latvia

The antifungal polyene antibiotics have been shown to mediate changes in permeability by forming ion-selective channels in lipid bilayers. The goal of this study was to investigate the polyene-induced conductance and ionic flux in cholesterol-containing muscle fibre membrane.

Cation conductance and efflux induced by polyene antibiotics amphothericin B (AMB), amphothericin B methyl ester (AME), amphothericin B ethyl ester (AEE), nystatin, mycoheptin, levorin, and roseofungin in frog isolated skeletal muscle fibres and whole sartorius muscles were investigated. Conductance was measured under current-clamp conditions using a double sucrose-gap technique. The potassium, rubidium, and sodium effluxes were studied using flame emission photometry.

Polyene antibiotics produced a concentration-dependent exponential increase in cation conductance to a steady-state that was reached in 6–8 min. The following order of polyene-induced conductance was obtained: levorin > AMB > mycoheptin > AME > nystatin. The induced efflux values for K^+ followed the order: levorin > AMB > mycoheptin > AME > AEE, nystatin, roseofungin. There was reverse temperature dependence of AMB- and nystatin-induced conductance. The decline in the equilibrium conductance caused by polyene removal (except for levorin) was very fast.

Our results seem to suggest that the steady-state characteristics of polyene complexes responsible for the permeability induction in biological membranes, and especially the kinetics of channel formation, might differ from those in model membranes.

O-682

Ionic channels in non-differentiated proliferative cells

B. Soria

Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Sevilla, Spain

Cancer cells and stem cells share many properties, including both self-renewal and multilineage differentiation. Ion channels are important for cell cycle progression and proliferation of melanoma and breast carcinoma cells. We have used mouse embryonic stem cells (mESC) as a model of a cell that proliferate and may be kept in a non-differentiated stage. Plasma membrane ionic channels: mESC posses L-type voltage-activated Ca2+ channels sensitive to nifedipine, small Ca2+-activated K+ channels sensitive to apamin. Blockade of L-type ca2+ channels by dihydropyridines inhibited cell proliferation, whilst apamin (a blocker of small Ca2+-activated K+ channels) has no effect on cell proliferation. Gap-junction channels: mESC establish gap-junction channels between pluripotent cells. Gap-junctional intercellular communication and connexin 43 plays a role in the proliferative and pluripotential capabilities of mESC. Defective intercellular communication, either by pharmacological blockers or by down-regulation using specific siRNA, induced a loss in their pluripotent state. Under these conditions the formation of embryoid bodies was impaired. Intracellular channels: mESC display receptors for many agonists present in the culture media. Activation of these receptors mobilizes Ca2+ from intracellular stores and promotes cell proliferation. An integrated interplay between all these components modify electrical properties and intracellular ion activities hence regulating mESC self-renewal and pluripotency.

P-683

The influence of pH and zinc ions on voltage-gated potassium channels in rat hippocampal neurons

A. R. Teisseyre, K. Mercik, J. W. Mozrzymas

Department of Biophysics; Medical University in Wrocław; ul. Chałubińskiego 10, 50-368 Wrocław, Poland

We applied the whole-cell patch-clamp technique to study the influence of extracellular pH (pH_o) and zinc ions (Zn) on the activity of voltage-gated potassium channels expressed in rat hippocampal neurons. We examined the total current and the delayed-rectifier component. In contrast to what was observed earlier in case of Kv1.3 channels in human lymphocytes, lowering of pH_o from the value of 7.35 to 6.4 did not affect significantly the currents. At pH_o = 6.4 the activation midpoint was shifted by about 6.3 mV (total current) and 6.4 mV (delayed-rectifier current) towards positive membrane potentials, but this shift was statistically insignificant. The current amplitudes and the current activation rate were not changed significantly by lowering the pH_o. Application of Zn in the concentration range from 100 μM to 5 mM shifted the activation midpoint of the currents by about 40 mV (total current) and 30 mV (delayed-rectifier current) towards positive membrane potentials and slowed significantly (2-3 fold) the current activation rate. The current amplitudes were reduced to about 0.5 of the control value upon application of 5 mM Zn. The modulatory effect of Zn both on the total current and on the delayed-rectifier component did not depend on the value of pH_o. The effect depended weakly on Zn concentration. Altogether, in contrast to what was observed in case of Kv1.3 channels in human lymphocytes, lowering of pH_o had no influence on the modulatory effects exerted by Zn on volatge-gated potassium channels in rat hippocampal neurons.

Abstracts

– Disordered and aggregated proteins –

P-684

Membrane surfaces acting as aggregation templates for amyloidogenic proteins: 2D versus 3D folding

C. Aisenbrey[1], R. Byström[1], T. Borowik[2], H. Misiak[2], F. Lindström[1], M. Bokvist[1], G. Gröbner[1]

[1]Department of Chemistry, Umeå University, Sweden, [2]Institute of Physics, Wrocław University of Technology, Poland

The pathological self-assembly of proteins plays a key role in amyloidogenic diseases. These proteins are prone to membrane-association and fibrillization due to their inherent structural H-bond packing defects. Using the membrane-mediated aggregation of Aβ protein as a model, we identify the general rules governing membrane-associated aggregation of proteins:

i) *Membrane surfaces as aggregation templates*: membrane association of proteins induces a surface crowding effect. In addition, protein diffusion is restricted to two dimensions, causing a much higher probability of protein-protein contacts. Above a critical surface concentration barrier, accelerated transformation of protein from monomeric to toxic aggregates can occur despite much lower bulk concentration.

ii) *Two factors, electrostatics and hydrophobicity*, are the major determinants of non-specific membrane binding. Association of proteins to specific membrane interfaces leads to an increased population of destabilized proteins resulting in a dramatic increase of amyloidogenic products. This way, we generate the necessary knowledge and methodological tools, to evaluate the physiological impact of membranes even for the demanding, soluble Cu-Zn superoxide dismutase 1 (SOD) system. Initial results by us suggest that misfolding and aggregation of SOD1 on membrane surfaces plays a role in amyotrophic lateral sclerosis (ALS).

P-685

Prevention of in vitro alcohol dehydrogenase thermal aggregation by the camel β-CN chaperone

A. Barzegar[1], A.-A. Moosavi-Movahedi[1], R. Yousefi[1], T. Haertle[2], A. A. Saboury[1], M. R. Ganjali[3], P. Norouzi[3]

[1]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, [2]Institut National de la Recherche Agronomique, Fonctions et Interactions des Prote´ines Laitie'res B.P. 71627, 44316 Nantes Cedex 3, France, [3]Department of Chemistry, College of Science, University of Tehran, Tehran, Iran

Molecular chaperones are a family of proteins which prevent protein aggregation under unfavorable conditions such as elevated temperature. Bovine caseins were shown recently to demonstrate chaperon activity in vitro. In this study camel β-casein (β-CN) was purified using a novel modified protocol, to investigate its chaperon activity. Chaperon activity of camel β-CN was examined, using UV-visible spectrophotometer by following aggregation of alcohol dehydrogenase (ADH) at 360 nm as target protein. Here the chaperon activity of camel β-CN was examined by us under thermal stress. Our results showed that the chaperon activity of camel β-CN was increased in a dose dependent manner and at the weight ratio of 1:1 it completely suppressed aggregation of ADH. The results also demonstrate that chaperon activity of this protein was sensitive to temperature, showing an inversely correlated to temperature elevation. Our results verify a temperature dependence chaperon activity for camel β-CN, as it has been reported for the well known chaperon such as GroEL and α-crystalline.

O-686

Molecular mimicry by an intrinsically unstructured protein

D. A. Bonsor[1], I. Grishkovskaya[1], E. J. Dodson[2], C. Kleanthous[1]

[1]Department of Biology, University of York, York, UK, [2]York Structural Biology Laboratory, Department of Chemistry, University of York, UK

Intrinsically Unstructured Proteins (IUPs) tend to be extended, highly dynamic polypeptides that undergo disorder-order transitions (-ΔH, -ΔS) as they form complexes with protein partners. An often-quoted characteristic of these binding interactions is their low affinity but high specificity. We have discovered a novel binding mechanism for an IUP, which we term "competitive recruitment", wherein a small disordered region in the bacteriocin Colicin E9 is able to outcompete a much larger globular protein (Pal) in binding to the *Escherichia coli* periplasmic protein TolB [1]. We report crystallographic and calorimetric data that reveal how the Colicin IUP accomplishes such recruitment [2]. Unusually, the IUP forms a high affinity complex with TolB ($K_d \approx 90$nM) by mimicking key hydrogen bonds made by Pal. Remarkably, the IUP also appears to block conformational changes in TolB that are induced by Pal. Our study demonstrates that small IUPs have the capability to compete with much larger globular proteins by mimicking their hotspot interactions with protein partners.

[1] Loftus *et al.* (2006) *Proc. Natl. Acad. Sci. USA* **103**, 12353

[2] Bonsor *et al*. (2007) *J. Am. Chem. Soc.* **129**, 4800

P-687

Structural disorder and induced folding within the replicative complex of measles virus

J.-M. Bourhis[1], V. Receveur-Bréchot[1], H. Darbon[1], V. Belle[2], A. Fournel[2], B. Guigliarelli[2], M. Oglesbee[3], S. Longhi[1]

[1]AFMB, UMR 6098 CNRS and Universities Aix-Marseille I and II, Marseille, France, [2]BIP, UPR 9036 CNRS, Marseille, France, [3]Dept. Veterinary Biosciences, The Ohio State University, Columbus, OH, USA

Measles virus (MeV) belongs to the *Paramyxoviridae* family within the *Mononegavirales* order. Its non segmented, single stranded, negative sense RNA genome is packaged by the nucleoprotein (N) within a helical nucleocapsid. This ribonucleoproteic complex is the substrate for both transcription and replication. The RNA–dependent RNA polymerase binds to the nucleocapsid template via its co-factor, the phosphoprotein (P).

In the course of the structural characterization of MeV replicative complex proteins, we discovered that the N and P proteins contain long disordered regions possessing sequence properties that typify intrinsically disordered proteins. We also reported induced folding of the intrinsically disordered C-terminal domain of N (N_{TAIL}) upon binding to the C-terminal domain of P (XD) and characterized at the molecular level this structural transition. We solved the crystal structure of XD and elaborated a model of the N_{TAIL}-XD complex. Finally, we showed that the XD-induced folding of N_{TAIL} is reversible. We propose a model where the dynamic breaking and reforming of the N_{TAIL}-XD interaction would allow the polymerase complex to cartwheel on the nucleocapsid template. The functional implications of structural disorder will be also discussed in light of the ability of disordered regions to establish interactions with multiple partners and hence trigger multiple biological effects.

Abstracts

– Disordered and aggregated proteins –

P-688

Understanding the role of protein context on polyQ aggregation

D. Bulone[1], L. Masino[2], D. J. Thomas[3], P. L. San Biagio[1], A. Pastore[2]

[1]CNR, Istituto di Biofisica, via La Malfa 153, I90146 Palermo, Italy, [2]NIMR, The Ridgeway, London, U.K., [3]Scientific Software Solutions, Orr Square Church, Paisley PA1 2DL, U.K.

Polyglutamine (polyQ) diseases are inherited neurodegenerative disorders caused by the expansion of CAG codon repeats, which code for polyQ in the corresponding gene products. These diseases are associated with the presence of amyloid-like protein aggregates, induced by polyQ expansion. The aggregation properties of polyQ are though to be strongly modulated by the surrounding protein context.

To assess the importance of the protein carrier in polyQ aggregation, we have studied the misfolding pathway and the kinetics of aggregation of polyQ of lengths above (Q41) and below (Q22) the pathological threshold fused to the well-characterized glutathione S-transferase (GST). This protein, chosen as a model system, is per se able to aggregate irreversibly, thus mimicking the behaviour of domains of naturally occurring polyQ proteins. We prove that, while it is generally accepted that the aggregation kinetics of polyQ depend on its length and are faster for longer polyQ tracts, the presence of GST alters the polyQ aggregation pathway and reverses this trend. Aggregation occurs through formation of a reservoir of soluble intermediates whose populations and kinetic stabilities increase with polyQ length. Our results provide a model which explains the toxicity of expanded polyQ proteins, in which the interplay between polyQ and other aggregation-prone domains plays a key role in determining the aggregation pathway.

P-689

Studies of the SARS-Coronavirus HR2 Domain in the Prefusion and Transition States

M. Caffrey, S. Hakansson, S. Jiang

University of Illinois at Chicago, U.S.A.

The envelope glycoprotein spike of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) is known to mediate viral entry. During viral entry, the heptad repeat domains of spike, termed S2-HR1 and S2-HR2, are thought to undergo structural changes from a prefusion state, in which S2-HR1 and S2-HR2 do not interact, to a postfusion state in which S2-HR1 and S2-HR2 interact to form a 6 helix bundle. In the present work, the biophysical properties of S2-HR2 in the prefusion state, as well as a newly discovered transition state, have been characterized. SEC and AU studies suggest that S2-HR2 is in a monomer-trimer equilibrium with the trimeric state stabilized by increased ionic strength or the presence of TFE, a co-solvent known to stabilize helical structures. Based on CD studies, S2-HR2 forms a helical structure in solution that is stabilized by increased ionic strength or the presence of TFE. NMR studies indicate that the helix core of S2-HR2 consists of residues 19-38. NMR dynamic experiments demonstrate the presence of an unstructured state, the transition state, that is in fast equilibrium with a structured state, the prefusion state. The structural properties of S2-HR2 prefusion and transition forms are discussed in the context of the postfusion form of S2-HR2. A model for viral entry is presented in which S2-HR2 is in a dynamic equilibrium between an unstructured transition state and a structured trimer in the prefusion state. Conversion from the prefusion state to the postfusion state requires passage through the transition state, a state that may give insight into the design of structure-based antagonists of SARS-CoV, as well as other enveloped viruses.

P-690

A computational study of pathogenic mutations in domain C5 of MyBPC

F. Cecconi[1], C. Guardiani[2], R. Livi[3]

[1]Istituto dei Sistemi Complessi (ISC-CNR), Roma, Italy, [2]Centro per lo Studio delle Dinamiche Complesse (CSDC), Università di Firenze, Italy, [3]Dipartimento di Fisica, Università di Firenze, Italy

The molecular pathogenic effect of three mutations of domain C5 of Myosin Binding Protein C (MyBPC) involved in Familial Hypertrophic Cardiomyopathy (FHC) is investigated through Molecular Dynamics simulations. The Phi-values are characterized by an asymmetric distribution and the mutation (Mut115) affecting the beta-sheet with the highest values of this indicator appears to be related to a more severe phenotype. The clinical impact of the mutations also correlates with their destabilizing effect and with the speed-up of the unfolding process. The analysis of contact probabilities, finally shows that the folding process proceeds along a specific direction, namely from the protein end close to Mut115, towards the area of Mut28. Mut115 thus hinders the onset of folding whereas Mut28 only interferes with the last stage of the process when the protein is already almost completely folded, thus explaining the different clinical phenotype of the two mutants. The destabilizing effect of the long CD loop featuring a 28-residue long insert peculiar of the cardiac isoform, was also investigated. The sequence analysis shows the typical signature of natively unfolded proteins: charge unbalance combined with low hydrophobicity. This suggests that the folding mechanism might be coupled with binding with a specific ligand, possibly domain C8 of MYBPC or the CaM-II-like kinase copurifying with MyBPC.

P-691

Characterizing unfolded proteins using nanopore detectors

Y. Cesa, V. Subramaniam, M. L. Bennink

Biophysical Engineering Group and MESA+ Institute for Nanotechnology, University of Twente, Enschede,The Netherlands

Nanopore detectors have provided a new approach to the characterization of biomolecules such as DNA and RNA. The biomolecules are driven by an electric field to and through a single pore. The translocation of the molecule can be detected measuring the current through the pore. Solid-state nanopores offer a number of clear advantages over biological pores, such as increased mechanical and chemical robustness, as well as size tunability. The size tunability gives the technique more flexibility showing a promise for characterizing proteins and other biomolecules in a broader range of sizes. We are exploring the potential of the nanopore technique to yield insight into the early stages of aggregation of intrinsically disordered proteins like α-synuclein. The deposition of these proteins in the form of amyloid fibrils and plaques is the characteristic feature of more than 20 degenerative conditions like Alzheimer's and Parkinson's. It is crucial to understand the mechanisms by which the aggregates are formed from their soluble precursors.

Abstracts

– Disordered and aggregated proteins –

P-692

Calcium binding regulates biophysical changes of the RTX domain of *Bordetella pertussis* CyaA toxin

A. Chenal, A. K. Wozniak, A. C. Sotomayor, J. I. Guijarro, M. Davi, C. Bauche, P. England, M. Delepierre, D. Ladant
DBSC, Institut Pasteur, CNRS URA 2185, Paris, France

Adenylate cyclase toxin (CyaA) is one of the major virulence factors of *Bordetella pertussis*, the causative agent of whooping cough. Its C-terminal region, the R domain, contains *ca.* 40 RTX (Repeat in ToXin) motifs, that are calcium-binding sequences characteristic of cytolysins produced by many pathogenic bacteria. Calcium is indeed an essential cofactor for CyaA binding to its receptor, the $\alpha_M\beta_2$ integrin, and subsequent entry into target cells.
To gain more insight into the structure-function relationship of the RTX motifs of the R domain of CyaA, we have characterised the conformational changes and the thermodynamic parameters of the transition from the apo- to the holo-state, using circular dichroism, fluorescence, NMR, intrinsic viscosity, dynamic and static light scattering. The results indicate that apo-R is mainly unfolded, highly solvated and adopts an elongated shape. Calcium binding induces the compaction and dehydration of R and the formation of stable secondary and tertiary structures. We propose that the unstructured character of the R domain in the absence of calcium may facilitate the secretion of CyaA by the dedicated type I secretion machinery. Upon reaching the extracellular milieu, calcium binding induces the folding of the R domain, thus providing a driving force for the secretion of the molecule. The calcium-induced conformational changes are also directly implicated in the recognition of the integrin receptor at the target cell surfaces.

P-693

Exploring the Energy Landscape of Human Lysozyme towards an Elucidation of Systemic Amyloidosis

A. Dhulesia, A. J. Baldwin, M. Dumoulin, J. R. Kumita, D. Nietlispach, X. Salvatella, C. M. Dobson
Departments of Chemistry and Biochemistry, University of Cambridge, U.K.

Human lysozyme systemic amyloidosis is a fatal disease associated with the deposition of proteic insoluble fibrillar aggregates. The formation of amyloid fibrils requires human lysozyme, natively folded, to undergo conformational changes leading to the population of aggregation prone species. An understanding of the fibrillization pathway and the interplay between the different species involved is essential for our ability to devise successful therapeutical approaches. We first focus on the low pH equilibrium between monomeric forms of human lysozyme and show how the unfolding reaction can be tuned using temperature and pH, as monitored by circular dichroism and tryptophan fluorescence, and rationalized by solution state NMR. Exchange NMR experiments have been carried out and should allow the rate constants for the folding and unfolding reactions to be derived. We provide evidence for the presence of a third amyloidogenic species, partially folded, which exhibits molten globular features: indeed, incubating monomeric lysozyme in vitro under conditions where this intermediate is significantly populated leads to the formation of amyloid fibrils, as characterized by a wide range of biophysical techniques which include circular dichroism, Thioflavin-T binding, electron microscopy and light scattering. Pulsed field gradient solution NMR techniques allow the measurement of the diffusion coefficient of those fibrillar aggregates. We further characterize the structure of the fibrils by using H/D exchange techniques directly on the amyloid fibrils.

P-694

The role of long and short range interactions on the multi-scale modeling of peptide oligomers

O. Engin, M. Sayar, B. Erman
Koc University, Istanbul, Turkey

The native structure of proteins is stabilized by both local and non-local interactions. The available phase space is highly reduced in size due to the local interactions, but non-local interactions determine the final physiologically active native structure. Although it is well-known that these two types of interactions are the key factors in determining the tertiary structure, their relative contributions is open for debate. This study will lay the groundwork for the investigation of relative contributions of these interactions. The contribution of both local and non-local neighbors to the Ramachandran map of the residue in question is examined by using statistical weight matrices (U) constructed according to the Markov assumption. An efficient matrix multiplication scheme based on rotational isomeric states model is introduced for studying realistic conformations of all- alanine, tryptophan, and valine short chains in the denatured state. This scheme is based on U's obtained from dipeptide and tripeptide simulations. Here, multi-scale modeling techniques are applied in order to apply the Markov model to all sequence lengths. Preliminary results suggest that the Markov assumption can be improved significantly by adding the contributions from hydrogen bonds and hydrophobic contacts, which are only present in the tetra-peptide sequence. Such a coarse-grained model will help elucidate the protein folding problem and improve secondary structure prediction algorithms.

O-695

The natively unfolded transcription activator Tat protein from HIV-1 is almost a random coil

M. Foucault[1], V. Receveur-Bréchot[2], C. Guillon[3], K. Mayol[3], R. Menduni[1], B. Verrier[3], R. Haser[1], P. Gouet[1]
[1]LBC, IBCP UMR 5086 CNRS UCBL, Lyon, France, [2]AFMB, CNRS, Marseille, France, [3]Laboratoire Immunothérapie des Maladies Infectieuses Chroniques, CNRS-BioMérieux, Lyon, France

A 101 residues long variant of the transcription activator Tat of the human immunodeficiency virus, Tat 133wt, has been chemically synthesized and its transactivity on the viral gene has been characterized. Structural investigations have been performed by computational, circular dichroism, gel filtration, dynamic light scattering and small angle x-ray scattering (SAXS) techniques. They reveal that Tat is a "natively unfolded protein". Notably, the atomic structures of Tat determined so far by NMR are not consistent with the scattering profile of Tat in solution. The dimensions of the protein assessed by SAXS and by dynamic light scattering, together with the SAXS Kratky plot, indicate that Tat likely exhibits a random coil state in solution, with very few residual structures. Only its small cystein-rich region which is conserved in all variants may adopt a stable conformation. This natively unfolded state is confirmed by an increase in the stability of the structure observed in the presence of trifluoroethanol or by complexing the protein with specific fragments of antibodies. The results observed here for Tat protein reveals that natively unfolded proteins cannot be classified under strict categories such as "molten globule", premolten globule" or "random coil". Rather there might exist a continuum of possible conformational states ranging from fully disordered with no residual interactions (random coil) to a molten-globule like state with more persistent secondary structures.

Abstracts

– Disordered and aggregated proteins –

P-696

Dynamics of disordered proteins as probed by time-of-flight neutron scattering: the case of caseins

A. M. Gaspar[2], W. Doster[1], M.-S. Appavou[1], S. Busch[1], T. Unruh[2]
[1]E13 - Physik Department, Technische Universität München, Germany, [2]FRMII - Forschungsneutronenquelle Heinz Maier-Leibnitz, Technische Universität München, Germany

Casein proteins belong to the class of natively unfolded proteins. The existence of disordered biologically active proteins questions the assumption that a rigid well-folded 3D-structure is required for functioning and forces a reassessment of the function structure paradigm. A hypothesis generally put forward is that the unstructured nature of these proteins results from the need of a higher flexibility.
In order to evaluate if there are significant differences in the internal picosecond dynamics of natively unfolded proteins with regards to that of proteins with a well defined three dimensional structure a series of time-of-flight neutron scattering experiments have been performed: on the three casein proteins, as well as on myoglobin (α-helical), concanavalin A (β-sheeted) and lysozyme (mixed α/β). To our knowledge these are the first dynamic neutron scattering experiments on disordered proteins, despite of the potential of the technique: the neutron's short wavelength gives us the spatial resolution necessary to probe the internal motions of proteins and by using D_2O, instead of H_2O, as solvent one can isolate the motions of the protein hydrogens, taking advantage of the fact the scattering power of the H atom is much higher than that of the other atoms.
Results obtained so far suggest, contrary to the initial expectation, slower localized side chain motions for the unstructured proteins. The effect of the solvent will be discussed.

P-697

Proline isomerization and oligomerization of alpha-synuclein studied by FCS and other techniques

M. Gerard[1], Z. Debyser[2], V. Baekelandt[3], Y. Engelborghs[1]
[1]Laboratory of Biomolecular Dynamics, University of Leuven, Belgium, [2]Laboratory for Molecular Virology and gene therapy, University of Leuven, Belgium, [3]Laboratory for Neurobiology and gene therapy, University of Leuven, Belgium

Fluorescence Correlation Spectroscopy (FCS) allows the determination of the average residence time and the size of fluorescently labeled molecules moving through a confocal volume. We used FCS to study alpha-synuclein (a-SYN) aggregation *in vitro*, a process that plays a key role in Parkinson's disease (PD). The mutant S42C a-SYN was specifically labelled with a thiol-reactive dye. Trace amounts of fluorescently labeled a-SYN were added to the wild type protein. With FCS, we could detect small oligomeric intermediates preceding the fibril formation. We also discovered that aggregation is accelerated by FK506 binding proteins (FKBPs), enzymes with rotamase activity. This could be counteracted by FK506, an inhibitor of FKBPs. Using Thioflavin T fluorescence and turbidity, we observed an effect of picomolar concentrations of human FKBP12 on the fibril formation of a-SYN. Electron Microscopy confirmed the fibrillar morphology of the aggregates. Using an enzymatically deficient FKBP12, we showed that the rotamase activity is responsible for the observed effect. FKBP12 did not have a pronounced effect on two disease mutants of a-SYN. We hypothesize that FKBPs accelerate the folding and subsequent aggregation of wild type a-SYN. FK506 and other non-immunosuppressive FKBP inhibitors display neuroregenerative and neuroprotective properties in PD models. Their ability to inhibit FKBPs and a-SYN aggregation may open new perspectives for the treatment of PD.

P-698

Isotope effect of the solvent on C-phycocyanin dynamics

G. Gibrat[1], S. Combet[1], M. Tehei[2], M.-C. Bellissent-Funel[1]
[1]LLB, Gif-sur-Yvette, France, [2]ILL, Grenoble, France

Heavy water (D_2O) is often used as a solvent for proteins in biophysics techniques, such as NMR or neutron scattering. However, some particular physicochemical properties of D_2O, as compared to light water (H_2O), can influence the behaviour of proteins, especially their dynamics.
The aim of the present study was to compare the dynamics of the C-phycocyanin (C-PC) in H_2O or D_2O. C-PC is a light-harvesting protein present in the cyanobacteria, which plays a key-role in the first steps of photosynthesis. This protein, which can be nearly fully deuterated, has also been hugely used as a protein model to study the dynamics of hydration water molecules at protein surface.
We measured on a backscattering neutron spectrometer (IN13, ILL, France) the mean square displacements ($\langle u^2 \rangle$, ∼100 ps resolution) of powders of C-PC hydrated in H_2O or D_2O (one monolayer of water molecules at the surface of the protein). Neutron scattering is a particularly powerful technique to study internal dynamics of a protein. The elastic spectra were analyzed with the "double-well" model described by Doster *et al.* [Nature 1989]. The mean square displacements of the C-PC hydrated in H_2O or in D_2O, as well as the associated thermodynamic values, were significantly different over all the range of temperature (20-320 K). Temperatures of the dynamical transition between harmonic and anharmonic behaviours were also different depending on the solvent used: 220 ± 10 K in H_2O and 270 ± 20 K in D_2O. Moreover, these results were confirmed by measurements of differential scanning calorimetry made on the same samples, which showed different transition temperatures between the C-PC hydrated in H_2O or D_2O.

P-699

Biophysical study of apo-calmodulin denaturation.

G. Gibrat[1], G. Hui Bon Hoa[2], Y. Blouquit[3], C. T. Craescu[3], M.-C. Bellissent-Funel[1]
[1]LLB (CEA-CNRS), Saclay, France, [2]Inserm, Le Kremlin-Bicêtre, France, [3]Institut Curie-Orsay, Orsay, France

Calmodulin is a calci-protein that can fix 4 calcium ions. There is two forms of calmodulin: the holo form (with calcium) and the apo form (without calcium). Apo form has a "dumbbell structure", with two N- and C-terminal domains, homologous of parvalbumin, linked by a central a-helix. Previous studies have found a two-steps denaturation pathway of apo-calmodulin: In the first step, around 48°C, the C-terminal domain unfolds, then, around 61°C, the N-terminal domain unfolds. We have performed circular-dichroïsm (CD) and fluorescence measurements in H_2O and D_2O, showing a 4°C shift of the two transitions. Apo-calmodulin depends thus on protein-solvent hydrogen bonding and hydration. We have then performed SANS measurements on PAXE spectrometer (LLB) showing synchronous evolution of the tertiary structure and of the secondary structure. This indicates that in unfolding pathway of calmodulin there is no "molten globule" state sufficiently stable to affect the transition curves. From 75°C, apo-calmodulin looses its structure and adopts a polymer-like conformation, with however some residual secondary structures. Thermal denaturation has been found to be irreversible, but, after a first hysteresis during the first heating-cooling cycle, it becomes reversible again: the native state of apo-calmodulin is so maybe a meta-stable state.We have also investigated the pressure denaturation of apo-calmodulin by fluorescence and SANS, showing a completely different behaviour: the pressure unfolding pathway contains a compact intermediate state around 3000 bar.

Abstracts

– Disordered and aggregated proteins –

P-700

Molecular mapping of the recognition interface between the Islet Amyloid Polypeptide and Insulin

S. Gilead, E. Gazit
Tel Aviv University, Tel Aviv, Israel

The self-assembly of endogenous proteins into amyloid fibrils is the hallmark of a group of diverse human disorders including Alzheimer's disease, Parkinson's disease, and type II diabetes. Many inhibition strategies have been developed in the attempt to prevent this key process. A leading approach is the search for natural entities that stabilize the native protein conformation and prevent amyloid formation in healthy individuals. Such a stabilizing agent might be insulin, in the case of the islet amyloid polypeptide (IAPP), which forms amyloid fibrils in the pancreas of type II diabetes patients. Insulin was shown to be an exceptionally potent inhibitor of IAPP fibrilization, and is thought to form a complex with IAPP within the pancreatic β-cells. Here, we investigated the molecular mechanism underlying the IAPP-insulin interaction. First, we compared the inhibition abilities of the A and B chains of insulin. Using ThT fluorescence and CD measurements, we found that the B chain, but not the A chain, is an excellent inhibitor of IAPP amyloid formation. Next, we mapped the entire B chain using consecutive overlapping peptide arrays and located a region that inhibits IAPP amyloid formation. We propose that this domain mediates recognition between IAPP and insulin. Moreover, by using a reciprocal assay, we located the interaction domain within IAPP as well. Interestingly, we found it to correspond to the IAPP self-recognition site. The molecular mapping of this interaction is of major importance in elucidating the mechanism underlying amyloid formation in type II diabetes. Moreover, it may be utilized for the design of peptidomimetic inhibitors of amyloid formation.

P-701

Concentration-temperature superposition of helix renaturation rates in gelatin

J. L. Gornall, E. M. Terentjev
Cavendish Laboratory, University of Cambridge, U.K.

Protein folding kinetics is crucial to understanding biologically active macromolecules. A number of experimental techniques have been used to study a great variety of natural and synthetic polypeptides undergoing globular collapse, or denaturation into the coil. Using optical rotation, combined with thermal characterization and rheological techniques, we have studied the kinetics of the helix-coil transition in gelatin. Gelatin is a protein derived from collagen by hydrolytic degradation. On cooling a gelatin solution from a denatured state a transition occurs to a transparent, thermoreversible gel containing extended physical cross-links. The cross-links are formed by partial renaturation of ordered triple helices, characteristic of the native collagen structure. In past studies, there has been a heated debated as to whether the concentration dependence of the helix renaturation rate is linear, quadratic or some other power. As a result of studying a much wider concentration range, we find the unexpected truth is an exponential dependence of renaturation rate on concentration. We also discover that the kinetics description can be dramatically simplified. Results from different concentrations and temperatures can be scaled onto a single Master curve using a new procedure we call the 'concentration-temperature superposition'. Remarkably, the form of the master curve is the same in water and ethylene glycol solutions of gelatin. There is a striking analogy with the classical time-temperature superposition in polymer physics, and the new time-concentration superposition in colloid glasses.

P-702

A novel gate in the helicase motor of SecA

G. Gouridis[1], L. Karamanou[2], T. Economou[2]
[1]University of Crete, 71409 Heraklion, Crete, Greece, [2]IMBB-FORTH, GR 711 10 Heraklion, Crete, Greece

SecA is the ATPase subunit of bacterial polypeptide translocase. SecA comprises an N-terminal ATPase domain (DEAD motor) containing motifs that are conserved in the DEAD-box Superfamily2 of helicases. The DEAD motor encompasses two domains, NBD (Nucleotide Binding Domain) and IRA2 (Intramolecular Regulator of ATPase). IRA2 binds to NBD and controls ADP release and optimal ATP catalysis (Karamamou *et al*, Sianidis *et al*). Two additional 'specificity domains' sprout out of the DEAD motor: the PBD (Preprotein Binding Domain) that is attached to NBD and the C-domain that is connected to IRA2. X-ray structural analysis (Papanikolau *et al*, in press) indicated that the DEAD motor is exclusively responsible for *E. coli* SecA dimerization. Specific NBD and IRA2 residues, previously identified from suppressor analysis of protein localization defects, (prlD; Huie *et al*) bind to each other in some SecA structures (Hunt *et al*, Sharma *et al*) and form a structure we term Gate 2. In the *ec*SecA dimer these residues are detached and hence Gate2 is "open" to allow dimer formation. In *ec*SecA dimer Gate2 residues of one protomer make contacts with the second protomer.
We now show that Gate 2 controls ATP hydrolysis and SecA dimerization. Gate 2 is required for high affinity ADP binding to SecA. Formation of the *ec*SecA dimer in the cytoplasm is compromised by Gate 2 mutations. We propose that preproteins may control SecA dimerization and ATP hydrolysis through Gate 2.

P-703

Effects of Cu(II) and Zn(II) on beta-lactoglobulin A denaturation and aggregation

R. Guzzi, A. Stirpe, C. Vecchio, L. Sportelli
Department of Physics - University of Calabria, 87036 Rende, Italy

Protein aggregation is associated with several neuro-degenerative diseases, including the Alzheimer's, the Parkinson's. There is a growing evidence that metal ions are able to accelerate the aggregation process of several proteins. In the present work the effects of copper and zinc ions on the denaturation and aggregation process of β-lactoglobulin A (BLG-A) are investigated by differential scanning calorimetry (DSC), optical density and fluorescence. The DSC profiles revealed that the thermal behaviour of BLG-A is a complex process consisting of an endothermic peak at about 80 °C (denaturation) and an exothermic peak above 90 °C which represents the aggregation of the denaturated molecules. Both the shape and the position of the two peaks are strongly dependent on the protein concentration and on the scan rate.
The presence of equimolar copper and zinc ions in the protein solution has different effects. In particular, copper is more effective than zinc in destabilizing BLG-A by decreasing the denaturation temperature of about 10 °C. The increase of the metals concentration reduces such a destabilization. This result may be due to the reduction of the net charge of the protein suggesting a central role of the electrostatics in the aggregation of BLG-A.
The kinetics of BLG-A aggregation, followed by monitoring the apparent absorbance of the protein solution at 400 nm, show that both metal ions abolish the lag phase before aggregationt. Moreover, the rate of the process is 4.7-fold higher when copper ion is added to the protein in a 1:1 molar ratio to the solution whereas the effect of zinc is negligible.

Abstracts

– Disordered and aggregated proteins –

O-704

The twilight zone between order and disorder

D. Györffy, A. Szilágyi
Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary

Intrinsically disordered proteins and protein segments often have a markedly different amino acid composition from that of ordered proteins. Here, our goal is to investigate the relationship between amino acid composition and order/disorder in more detail. First, we analyzed the order/disorder behavior of model proteins. Two types of 2D lattice models were used: the conventional hydrophobic-polar (HP) model and a model employing three monomer types: hydrophobic and positively and negatively charged monomers. We generated all possible sequences for chains up to 24 residues in length, and determined their ground state energies by enumeration. For longer chains, sequence space was sampled and the energies were approximated. Sequences with a ground state energy per monomer higher than a threshold were considered disordered. Looking at the relationship between amino acid composition and order, we found a chain length dependent transition from disorder to order in both models. For chains with a given length, there is a well-defined region in amino acid composition space where most sequences are ordered, and another region where most are disordered. Between these two regions, there is a "twilight zone" with sequences that can be ordered or disordered, depending on the specific sequence. Both the width and the position of the twilight zone depend on chain length. Turning to real proteins, we demonstrate the existence of the twilight zone on charge-hydrophobicity plots, and show that both the width and the position of the twilight zone depends on protein length in accordance with the theoretical predictions.

P-705

A disordered intermolecular protein binding epitope is also an intramolecular binding epitope

O. Hecht[1], R. Boetzel[1], C. MacDonald[1], H. Ridley[2], J. H. Lakey[2], G. R. Moore[1]
[1]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK, [2]Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle NE2 4HH, UK

Colicins are proteins secreted by *E. coli* to kill other bacteria. A common characteristic of colicins is a 3-domain structure: one for recognising target cells (R-dom), another for killing them (K-dom), and the third, the translocation domain (T-dom), for getting the K-dom into the target. Another common characteristic is that the T-dom is at least partially disordered. The best characterised colicin family in this respect are the 61 kDa E-type nuclease colicins (E2-E9). The T-dom residues that form the binding site for a receptor protein in target cells, TolB, have some order in the absence of TolB with side chains interacting with each other in a manner that restricts the motion of the backbone detected by ^{15}N NMR R_2 measurements. These clusters of side chains are disrupted by binding TolB. The 43 kDa pore-forming colicin N has no structural similarity with the nuclease E-type colicins beyond having a 3-domain structure and disordered T-dom, and enters target cells by a different route to E-type colicins, binding to TolA. The poster will present NMR data for colicins E9 and N which show their disordered T-doms have different dynamics, probably because the T-dom of ColE9 is independent of its R-dom and K-dom while the T-dom of ColN interacts intramolecularly with the TolA binding epitope of ColN binding to a structured region of ColN. This self-recognition will interfere with rate enhancement for TolA binding via the fly-catcher mechanism but may be advantageous for stability.

P-706

Simulation Studies of the Lipid-Dependent Structure and Aggregation Propensity of apoC-II (60-70)

A. Hung[1], M. Griffin[2], G. Howlett[2], I. Yarovsky[1]
[1]School of Applied Sciences, RMIT University, GPO Box 2476V, Melbourne VIC 3001 Australia, [2]Bio21 Molecular Science and Biotechnology Institute, 30 Flemington Rd, Parkville VIC 3010 Australia

Human apolipoprotein C-II (apoC-II) serve important roles in lipid transport, and has been shown to form amyloid-like aggregates in solution. Recent studies have demonstrated that, in addition to the full-length protein, a peptide fragment composed of residues 60-70 also have a propensity for amyloid fibril formation in solution. However, the presence of phosphatidylcholine (PC) lipids at sub-CMC inhibits fibrillisation. In this work, we employ molecular dynamics (MD) simulations to study the structures and aggregation propensities of the apoC-II (60-70) peptide fragment in water and in dilute PC-water mixtures in order to determine the mechanisms of initial peptide aggregation in solution, as well as the influences of lipids on inter-peptide interactions. Specifically, we examine the peptide conformations sampled in equilibrium and non-equilibrium MD trajectories in water and in PC solutions; the interactions between peptide and PC; and the manner in which PC alters the structure and dynamics of the peptide and vice versa. Furthermore, we use non-equilibrium MD methods to calculate PC-dependent peptide dimerisation free energies and the conformations sampled during dimerisation. These results elucidate the effects of lipid interactions on peptide structures and aggregation energies, and lend insights into their possible inhibitory effects on fibril formation.

P-707

Role of the Monomer and Oligomer in the Interaction of Islet Amyloid PolyPeptide with Membrane

L. Khemtémourian[1], M. F. Engel[2], J. W. Höppener[2], B. de Kruijff[1], A. J. Killian[1]
[1]Biochemistry of Membranes, Utrecht University, Netherlands, [2]Metabolic and Endocrine Diseases, University Medical Center Utrecht, Netherlands

Amyloid deposits in the pancreatic islets of Langerhans are thought to be a main factor responsible for death of the insulin-producing islet β-cells in type 2 diabetes mellitus. It is hypothesized that β-cell death is related to the interaction of the 37 aa residue human islet amyloid polypeptide (hIAPP) with cellular membranes. However, the conformational and aggregational state of the membrane-bound form has not yet been well characterized. Here we study i) the membrane interactions of IAPP and ii) the structure of membrane interacting IAPP. Our results show that hIAPP and the non-cytotoxic and non-amyloidogenic mouse IAPP (mIAPP) insert into phospholipid monolayers, whereas fibrillar hIAPP does not insert. In addition, we show using a quantitative membrane leakage assay that hIAPP causes membrane leakage with a lag time of several minutes, while fibrillar hIAPP and mIAPP do not cause leakage. These combined results suggest that oligomers are involved in inducing membrane damage. The results by circular dichroism in TFE (membrane mimicking environment) show that hIAPP initially adopts an α-helical, before transforming into a β-sheet structure. The β-sheet intermediate in hIAPP fibril formation is proposed to play an important role in membrane binding and damage. Consisting with this, CD spectra show that mIAPP adopts a stable α-helical structure. Our results suggest that hIAPP monomers insert into the membrane as an α-helical structure and that next hIAPP oligomers form are responsible for damaging the membrane most likely as a β-sheet structure.

Abstracts

– Disordered and aggregated proteins –

P-708

Dimerization of amyloidogenic 12-residue sequence of the adenovirus fiber

M. Kittner, V. Knecht
Max Planck Institute of Colloids and Interfaces, Department of Theory and Bio-Systems, Research Campus Golm, Am Muehlenberg 1, 14476 Potsdam, Germany

The development of specific agents against amyloidoses requires an understanding of the early stages of fibril nucleation on the microscopic level. We have used molecular dynamics simulations to study the dimerization of a 12-residue fibrillogenic peptide corresponding to residues 381-392 (LSFDNSGAITIG) of the human adenovirus type 2 fiber in atomic detail and explicit solvent. Previous simulations in our group predict a β-hairpin structure for monomers. In simulations of two peptides, dimerization is initiated by hydrophobic interactions and tends to destabilize the β-hairpin structure. Associated with the formation of intermolecular β-sheets, the β-sheet content of residues 382-383 increases by a factor of six. Residue contacts important for the dimerization process are identified. Ordered dimers typically arise from β-sheet extension or stacking.

P-709

Phosphorous dendrimers as antiprion agents

B. Klajnert[1], M. Cortijo-Arellano[2], J. Cladera[2], J. P. Majoral[3], A. M. Caminade[3], M. Bryszewska[1]
[1]Department of General Biophysics, University of Lodz, Poland, [2]Center of Studies in Biophysics, Universitat Autonoma de Barcelona, Spain, [3]Laboratoire de Chimie de Coordination, CNRS Toulouse, France

Inhibition of fibril assembly is a potential therapeutic strategy in prion diseases. Dendrimers - a novel group of polymers are promising antiamyloid agents. It has been shown that dendrimers possessing cationic amino groups on the surface are able to eliminate PrP^{Sc} from scrapie-infected neuroblastoma cells and inhibit the infection [1]. Further studies showed that the same dendrimers inhibited the aggregation of prion and Alzheimer's peptides [2].
The effect of cationic phosphorous dendrimers on the aggregation process of the prion peptide PrP 185-208 was studied using an spectrofluorometric assay with thioflavin T and Fourier transformed infrared spectroscopy in order to monitor the kinetics of the process and the changes in the peptide secondary structure. The results show that phosphorous dendrimers are able to interfere with PrP 185-208 aggregation process by both slowing down the formation of aggregates (by causing a decrease of the nucleation rate) and by lowering the final amount of amyloid fibrils, a common hallmark of conformational diseases.
[1] Supattapone, S. Nguyen, H.-O. B., Cohen, F. E., Prusiner, S. B. & Scott, M. (1999) Proc. Natl. Ac. Sci. 96, 14529
[2] Klajnert, B., Cortijo-Arellano, M., Cladera, J. & Bryszewska, M. (2006) Biochem. Biophys. Res. Commun. 345, 21

P-710

Chaperone activity of ERD10 and ERD14, two intrinsically unstructured stress-related plant proteins

D. Kovacs[1], E. Kalmar[2], P. Tompa[1]
[1]Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Hungary, [2]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Hungary

ERD10 and ERD14 (early response to dehydration, ERD) proteins are members of the dehydrin (DHN) family which accumulate in response to abiotic environmental stresses, such as high salinity, drought and low temperature, in *Arabidopsis thaliana*. Whereas these proteins protect cells against the consequences of dehydration, the exact mode(s) of their action remain poorly understood. Here we provide detailed evidence that these proteins belong to the family of intrinsically unstructured proteins (IUP), and we demonstrate in various assays that these proteins act as chaperones *in vitro*. ERD10 and ERD14 are able to prevent the aggregation and/or inactivation of various substrates, such as alcohol dehydrogenase, firefly luciferase and insulin, and promote reactivation of denatured and reduced lysozyme. We also demonstrate that these ERDs bind to acidic phospholipid vesicles (PLV), without significantly affecting membrane fluidity, though. Membrane binding is strongly influenced by ionic strength. These results show that these IUPs have a chaperone activity of rather wide substrate specificity, and they interact with phospholipid vesicles through electrostatic forces. We suggest that these findings provide the rationale for the mechanism of how these proteins avert the adverse effects of dehydration stresses.

O-711

Inferring protein function from patterns of native disorder

A. Lobley[1], M. B. Swindells[2], C. A. Orengo[3], D. T. Jones[1]
[1]Bioinformatics Unit, Department of Computer Science, University College London, Gower Street, London WC1E 6BT, UK, [2]Inpharmatica Ltd, 1 New Oxford Street, London WC1A 1NU, UK, [3]Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK

In this study we directly address the potential contribution of protein disorder in predicting protein function using standard Gene Ontology categories. We have analysed the occurrence of protein disorder in the human proteome and report ontology categories that are enriched in disordered proteins. Furthermore, pattern analysis of the distributions of disordered regions in human sequences demonstrated that the functions of intrinsically disordered proteins are both length and position dependent. These dependencies were then encoded in feature vectors to quantify the contribution of disorder in human protein function prediction using SVM classifiers.
The prediction accuracies of 26 GO categories relating to signalling and molecular recognition are shown to be were improved using the disorder features. The most notable improvements were observed for kinase, phosphorylation, growth factor and helicase categories. Furthermore we have generated predicted GO term assignments using these classifiers for un-annotated and "orphan" human proteins. Finally, we have benchmarked our method against a similar method for recognising protein function from sequence and report improved classification performance for all tested Molecular Function and Biological Process GO terms.

Abstracts

– Disordered and aggregated proteins –

P-712

Lipid dependant disorder-to-order conformational transitions in apolipoprotein CI derived peptides

P. Mendoza-Espinosa[1], A. Moreno[2], R. Castillo[3], J. Mas-Oliva[1]
[1]Instituto de Fisiología Celular, [2]Instituto de Química, [3]Instituto de Física, Universidad Nacional Autónoma de México, Mexico

In contrast to the notion established for many years that protein function depends on rigid 3D structures, nowadays there is important evidence suggesting that non-structured segments of proteins play important roles in protein function. Therefore, disorder-to-order dynamic conformational transitions have been proposed as an attractive mechanism involved in protein-protein recognition. Our laboratory previously used Langmuir monolayers of apolipoproteins to show that at the air/water interface, upon lateral compression, there is an important movement of specific segments of these apolipoproteins towards the air. More recently, apolipoprotein CI placed at phospholipid/water interfaces has also shown that with an increase in lateral pressure, the C-terminal segment moves towards the acyl-chain region of the interface. Here, in an attempt to define the secondary structure changes that might occur with this C-terminal segment of apoCI while moving from the hydrophilic interface towards hydrophobic regions, employing three peptides derived from the complete sequence of apoCI, we studied by circular dichroism and dynamic light scattering their conformational properties when associated to a series of amphipathic lipids and lipid-like molecules. The results found with a series of lysophospholipids, raise the possibility that specific segments of apoCI at the surface of high density lipoprotein particles, following lipid dependant disorder-to-order conformational transitions might modulate protein function of lipoprotein associated enzymes as well as lipid transport.

P-713

The role of fibril breakage in the amyloid propagation of β2-microglobulin

A. Micsonai[1], É. Petrik[1], L. Gráf[1], H. Yagi[2], Y. Goto[2], J. Kardos[1]
[1]Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary, [2]Institute for Protein Research, Osaka University, Osaka, Japan

Recent progress in the field of protein aggregation suggest, that the classical view of amyloid fibrils being irreversibly formed, highly stable structures resistant to heat and proteolytic digestion, is getting more colorful. We studied the structural stability and propagation of amyloid fibrils of β_2-microglobulin (β2m), a protein responsible for dialysis-related amyloidosis. By heat treatment, β2m fibrils can be dissociated to monomers in a reversible and dynamic process involving two distinct steps: (1) dissociation of monomers from the fibril ends and (2) the breakage of fibrils into pieces. We found that the fracture of β2m fibrils occurs even at physiological temperatures without strong mechanical stress such as sonication. In the process of amyloid propagation, new fibrils may be formed by spontaneous nucleation and/or by breakage of pre-formed fibrils. In polymerization experiments, under appropriate conditions, we were able to exclude spontaneous nucleation and exclusively study the increase of the extendable fibril ends by breakage. This process might have *in vivo* importance in amyloid propagation where the small number of fibrils, grown from spontaneously formed nuclei, is multiplied by breakage of longer fibrils resulting in a rapid growth after a lag phase. Thioflavin T fluorescence assay was used to measure the growth rate and fibril content in the protein solution. Morphology and length of fibrils were studied by electron microscopy. Our results are in line with theoretical predictions and might be beneficial in working out strategies to prevent or treat amyloid diseases.

P-714

Role of metals in the process of amyloid beta peptide polimerization

V. Minicozzi[1], S. Morante[1], F. Stellato[1], M. Dalla Serra[2], C. Potrich[2], W. Meyer-Klaucke[3]
[1]Università di Roma "Tor Vergata" INFM & INFN, Roma, Italy, [2]ITC & CNR-IBF, Trento, Italy, [3]EMBL, DESY, Hamburg, Germany

Alzheimer's disease (AD) is a neurodegenerative pathology characterized by memory disorders accompanied by senile plaque formation, amyloidosis of brain vessels and intraneuronal deposits of amyloid fibrils. AD plaques are mainly composed by β-amyloid peptides (Aβ) and contain large amounts of Cu and Zn. The latter is more effective as an aggregation promoter than the former, which has instead an inhibitory effect on Zn^{2+} induced aggregation. The existence of two metal-peptide binding modes have been suggested: an inter-molecular mode where Aβ-peptides are cross-linked by Aβ–metal–Aβ bridges and an intra-molecular one where metal coordinated atoms all belong to the same peptide. X-ray Absorption Spectroscopy has been used to study the local structure around the metal in $A\beta_{1-40}$ and shorter peptides complexed with Cu or Zn ions, and we recognized two different structures according to the complexed metal ion. The geometry around Cu is consistent with an intra-peptide binding, while different sample preparations lead to different geometries around Zn^{2+} ions, compatible with either an intra- or an inter-peptide coordination mode. The results reinforce the hypothesis that assigns different physiological roles to the two metals, with Zn favouring peptide aggregation and thus plaque formation, and studies performed on shorter peptides have allowed to locate the metal binding site within the first few residues.

P-715

Conformational states of the sequence Met1-Val60 of N-terminus of tyrosine hydroxylase

N. N. Mustafayeva
Baku State University, Azerbaijan

Tyrosine hydroxylase (TH) is a key enzyme of catecholamine biosynthesis. TH is regulated by a combination of reversible phosphorylation of serine residues in the regulatory domain and binding of catecholamines to the active site in the catalytic domain. The regulatory domain has been assign to the N-terminal end, and has an important role in substrate specificity and in control of the enzyme activity. At present there is no information regarding the three dimensional structure of the regulatory domain of this enzyme or how it is modulated by phosphorylation. In this study the molecular modeling methods were used to examine the three dimentional structure and conformational parameters of the specific portion from residue 1 to residue 60 in regulatory domain of TH. The fundamental assumption is that the native structure of the such polypeptide chain corresponds to the conformation which has the lowest energy. Two computational methods were used to solve the problem- method of molecular mechanic designed to give accurate structures and energies of the polypeptide chain, and the method of molecular dynamics, the computational method for simulating the motion of a system of many particles. The intrinsic energy of a polypeptide chain also includes the torsion potensials describing the barriers of the inner rotation between atoms that have a 1-4 relationship. The energy of hydrogen bond formation was calculated based on the Morse potential and the dissociation energy of the hydrogen bond was taken to be -1.5 kcal/mol at an NH...OC distance of r_0=1.8 A. The energy minimization was repeated until the minimal values of the global energy remained at a constant level.

Abstracts

– Disordered and aggregated proteins –

P-716

Initial folding events detected in an unfolded protein show why it forms a misfolded intermediate.

S. M. Nabuurs, A. Westphal, C. P. van Mierlo
Wageningen University, Laboratory of Biochemistry, Netherlands

The topology of the 179-residue Azotobacter vinelandii apoflavodoxin is characterised by a five-stranded parallel β-sheet surrounded by α-helices. During denaturant-induced equilibrium unfolding of apoflavodoxin a stable folding intermediate is populated. Kinetic folding experiments show that this intermediate is off the productive folding pathway and acts as a trap and that 90% of the folding molecules pass through this misfolded intermediate. In addition, all folding apoflavodoxin molecules pass through a high-energy on-pathway intermediate before reaching the native state. Understanding the conformational features of the unfolded state of apoflavodoxin is required to explain why the misfolded off-pathway intermediate is formed. We have structurally and dynamically characterised unfolded apoflavodoxin by using heteronuclear NMR spectroscopy. All backbone resonances of the unfolded protein have been assigned in 3.4 and in 6.0 M GuHCl. Chemical shift changes upon lowering the concentration denaturant from 6.0 to 3.4 M reveal that several regions of the unfolded protein form helices. 15N Relaxation studies show elevated relaxation rates in these α-helical regions at 3.4 M GuHCl as compared to the unstructured protein in 6.0 M GuHCl. Two of the three helical regions detected are also helical in native apoflavodoxin. However, β-strand 3 of native apoflavodoxin forms a helix in unfolded apoflavodoxin at 3.4 M GuHCl. The formation of this non-native helix is a plausible explanation for the formation of the misfolded off-pathway intermediate that is detected during the kinetic folding of apoflavodoxin.

P-717

Unbiased cold denaturation: low and high temperature unfolding of yeast frataxin under physiological

A. Pastore[1], S. R. Martin[1], P. A. Temussi[2]
[1]NIMR, MRC, Mill Hill, London, UK, [2]University of Naples, Naples, Italy

Temperature unfolding of proteins can occur at temperatures both higher and lower than room temperature, generally called "heat denaturation" and "cold denaturation" respectively. Accurate analysis of heat and cold denaturation processes promises to unveil hitherto obscure aspects of protein stability and dynamics. However, since the cold denaturation temperature of most proteins occurs well below the freezing point of water, the full access to the cold denatured state is normally limited for the obvious reason that water freezes at 0°C. The most common approach to circumvent this difficulty has been to try to raise the temperature of cold denaturation, using destabilising agents such as extreme pH values, chemical denaturants, cryosolvents or very high pressure. Alternatively, several laboratories have destabilized proteins by point mutations, The main drawback of these approaches is that it is not generally possible to extrapolate the results to physiological conditions.
Following a different approach, we looked for a protein whose cold denaturation could be studied in a normal buffer at physiological pH without the need of destabilization, in a temperature range accessible to several techniques. Here we describe the cold and heat denaturation of yeast frataxin (Yfh1), measured both by NMR and CD spectroscopies. We show that Yfh1 is a valuable protein to study low and high temperature transitions in a range accessible to many techniques, and thus it is an excellent model system which holds the promise to reveal new insights of the influence of several parameters on protein unfolding.

P-718

Biophysical studies of the conformational effects of proteolysis of the prion protein

O. Polyakova[1], A. Nash[1], S. Bawumia[1], P. Bayley[1], G. Dodson[2], I. Bronstein[1]
[1]Div. Physical Biochemistry NIMR London UK, [2]Div. Molecular Sructure NIMR London UK

The prion protein, Prp, is implicated in neuropathogenic prion diseases, that include the transmissible Bovine Spongiform Encephalopathy (BSE) in cattle and Creutzfeldt-Jakob Disease (CJD) in humans (1). They are related to the amyloid diseases (including Alzheimer's, Huntington's and Parkinson's diseases) in being characterised by the intracellular accumulation of a specific protein as an aggregated and often fibrillar form (2). The biophysical interest in Prp involves the transition from its normal membrane-bound conformation (∼ 35% α-helix) into conformers that are enhanced in β-structure, and which appear as aggregates and fibrils. In vivo, the protein undergoes proteolysis in the course of intracellular trafficking. The proteasomal system has a limited capacity for removal of abnormally folded forms, which, in excess, give rise to typical intracellular deposits. We report the unexpected conformational consequences of in vitro proteolysis of ovine Prp (ARQ allele: 94-231), (3), studied by CD spectroscopy, and supramolecular assembly monitored by electron microscopy and thioflavin fluorescence. We relate the findings to published studies of pathological consequences of the in vivo (transgenic) expression of variants of the prion protein. (1) http ://users.rcn.com/jkimball.ma.ultranet /Biology Pages/P/Prions.html (2) Uversky VN, Fink AL (2004) Biochim.Biophys.Acta 1698, 132-153 (3) Haire LF et al., (2004) J.Mol.Biol. 336, 1175-83. Supported by DH70106 and MRC UK

P-719

Structural impact of heparin binding to full-length neuronal Tau involved in Alzheimer disease

N. Sibille[1], A. Sillen[1], A. Leroy[2], J.-M. Wieruszeski[1], B. Mulloy[3], I. Landrieu[1], G. Lippens[1]
[1]CNRS UMR 8576, Université de Lille 1, France, [2]Laboratoire de biochimie appliquée, Paris XI, France, [3]National Institute for Biological Standards and Control, Potters Bar, U.K.

The neuronal Tau protein -involved in stabilizing microtubules- is the major component of the paired helical filaments (PHFs), intracellular aggregates characterizing Alzheimer's disease (AD) in neurons. The formation of AD-like aggregates by Tau can be induced *in vitro* by adding polyanions such as heparin. The microtubule binding repeats (MTBRs) have been identified as the key component in Tau aggregation, but the fact that the full-length protein does not aggregate by itself indicates the presence of intrinsic inhibitory factors. Positively charged regions flanking the MTBR have been recently proposed as the inhibitor of PHF assembly. Hyperphosphorylation of Tau could then neutralizes these basic inhibitory domains, enabling Tau-Tau interactions. We present the results of an NMR study describing the interaction between intact full-length Tau and small heparin fragments. Charge neutralization due to heparin binding, well mimics Tau hyperphosphorylation. Three-dimensional heteronuclear NMR experiments demonstrate that the interaction with heparin induces b-strand structure in several regions of Tau -that might act as nucleation sites for its aggregation- and indicate as well a-helical structure in regions outside the core of PHF. We propose that heparin becomes integrated into the rigid core region of the PHF, probably providing the charge compensation for inter-molecular polylysine peptide interaction that form the fibbers by parallel in-register stacking.

Abstracts

– Disordered and aggregated proteins –

P-720

Aggregation of fragments of Islet amyloid polypeptide as a phase transition: clustering analysis

G. Singh, I. Brovchenko, A. Oleinikova, R. Winter
Department of Chemistry, Biophysical Chemistry, University of Dortmund, Germany

Amyloid polypeptides are weakly soluble in water and even at low concentrations their aqueous solutions undergo phase separation onto two phases with peptide-rich phase beeing an ordered solid phase (fibrils). Studies of fibril formation can be done by the simulations of a peptide-water mixture with peptide concentration deeply inside a two-phase region. In such states, various system properties, including clustering (aggregation) of molecules, is extremely sensitive to the system size [1]. In particular, small size of the simulated system may prevent phase separation. We have performed MD simulations of the aqueous solutions of amyloidogenic fragments of IAPP (residues 15-19) at various concentrations (60 to 120 mg/mol) and various numbers of peptides (6 to 56). In each simulated, initial configurations were prepared by random placement of peptides in the simulation box. Clustering and aggregation of the peptide was studied by using interpeptide hydrophobic contacts or hydrogen bonds as connectivity criteria. Analysis of the probability distribution of the peptide cluster size allows distinguishing the states of the simulated system, where peptide-rich phase is absent or present. Only in the latter state the system may show behavior, relevant to the behavior of the macroscopic system at the same peptide concentration. We propose to use analysis of the peptide clustering to select the configurations relevant for larger systems and, therefore, for experiment.

[1] L.G.MacDowell, P.Virnau, M.Mueller, K.Binder, *J. Chem. Phys*., **120**, 2004, 5293

P-721

Preferential hydration of lysozyme in water/glycerol mixtures. Small-angle neutron scattering study

R. Sinibaldi[1], M. G. Ortore[1], F. Spinozzi[1], F. Carsughi[1], H. Frielinghaus[2], S. Cinelli[3], G. Onori[3], P. Mariani[1]
[1]Dipartimento di Scienze applicate ai Sistemi Complessi, Università Politecnica delle Marche, Ancona, Italy, [2]Institut fur Festkorperforschung, Forschungszentrum, Jülich, Germany, [3]Dipartimento di Fisica, Università di Perugia, Italy

In solution Small Angle Neutron Scattering has been used to study the solvation properties of lysozyme dissolved in water/glycerol mixtures. To detect the characteristics of the protein-solvent interface, 35 different experimental conditions (i.e., protein concentration, water/glycerol fraction in the solvent, content of deuterated compounds) have been considered and a suitable software has been developed to fit simultaneously the whole set of scattering data. The average composition of the solvent in close vicinity of protein surface at each experimental condition has been derived. In all the investigated conditions, glycerol resulted especially excluded from the protein surface, confirming that lysozyme is preferentially hydrated. Considering a thermodynamic hydration model based on an equilibrium exchange between water and glycerol from the solvation layer to the bulk, the preferential binding coefficient and the excess solvation number have been estimated. Results were compared with previous data for ribonuclease A also dissolved in water/glycerol mixtures: even if the investigated conditions were very different, the agreement is noticeable. Moreover, the excess solvation number shows as a function of solvent composition a maximal hydration region, which probably accounts for the changes in protein stability detected in the presence of co-solvents.

P-722

Thermodynamic analysis of insulin aggregation and amyloid formation

V. Smirnovas, R. Winter
University of Dortmund, Department of Chemistry, Physical Chemistry I – Biophysical Chemistry, Otto-Hahn Str. 6, Dortmund, Germany

In this work, a thorough thermodynamic study of the amyloidogenesis of insulin has been performed which provides a coherent and precise description of the changes of the partial specific volume, heat capacity, the coefficient of thermal expansion, as well as the adiabatic and isothermal compressibility of the protein upon partial unfolding, aggregation and fibril formation. This was only possible due to a novel application of ultrasound velocimetry and pressure perturbation calorimetry, complemented by differential scanning calorimetry, FT-IR spectroscopy and atomic force microscopy studies. Based on the data obtained, we are putting forward a comprehensive discussion of the aggregating and fibrillation process of the protein that may link its thermodynamics to the generic behavior of proteins implicated in conformational diseases (such as Alzheimer, Parkinson and Diabetes mellitus) in general.

P-723

Colorimetric biosensor for studying the molecular mechanism of amyloid protein-membrane interactions

M. Sokolovski, S. Kolusheva, R. Jelinek
Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Formation of amyloid fibrils is a common characteristic of a variety of unrelated diseases. A partial list includes Alzheimer's disease, type II diabetes, Parkinson's and others. Amyloid fibrils appear to share a common characteristic of highly ordered filaments with cross-β-sheet structure that forms the core of the fibrils, even though the amyloid fibrils proteins have no obvious amino acid sequence similarity. It was proposed that amyloid-forming proteins exert their toxicity via common primary step involving interaction of the aggregates within cell membrane that eventually leads to disintegration of the lipid bilayer.

In order to investigate the molecular mechanism of amyloid protein interactions with lipid membrane we designed a novel colorimetric biosensor, composed of conjugated polydiacetylene (PDA) matrix embedded within lipid bilayer. The system is capable of detecting various biological events occurring in lipid membrane through rapid colorimetric as well as fluorescent transitions. The colorimetric lipid assay, vesicle topographies and films at the air-water interface, has been applied to analyze membrane association of the various amyloid proteins, such as Casein and Insulin. The colorimetric response of the sensor is correlated to extent of protein-lipids binding and permeation and provides quantitative evaluation of membrane–active species among different aggregation stages of the proteins. The results obtained by PDA-based system were confirmed by different techniques: transmission electron microscope (TEM), dyes such as thioflavin T (ThT) and circular dichroism (CD).

Abstracts

– Disordered and aggregated proteins –

P-724

New flow/x-ray scattering methods for the analysis of amyloid and other fibrous aggregates

A. M. Squires[1], C. M. Dobson[2]

[1]University of Reading, U.K., [2]University of Cambridge, U.K.

Fibrous proteins and aggregates make up the majority of the biomass of the natural world. They include β-sheet "amyloid" fibrils that are the subject of much current research, due to their association with a number of diseases. Fibrous proteins are not amenable to structural characterization by conventional techniques used for globular proteins, such as x-ray crystallography or solution NMR. Other techniques are therefore required in order to obtain structural information on such systems.

Much of our knowledge of amyloid fibril structure is obtained using fiber-averaged wide-angle x-ray scattering (WAXS) carried out on dried, aligned samples. Our current model is of a "cross-β" structure, where the peptide chains assemble into β-sheets, with the constituent β-strands running perpendicular to the fibril axis, and where a number of these sheets stack together in the core of the fibril. These give rise to a WAXS pattern showing an axial reflection at 4.7 Å from the inter-strand spacing, and an equatorial reflection, typically at 8-12 Å, showing the spacing between sheets.

It has been suggested, however, that some of these features may be artefacts induced by the drying processes used in sample preparation. We have therefore designed a WAXS cell that aligns sample solutions by flow, allowing fiber-WAXS patterns to be obtained without sample dehydration. Data from diluted and dried aligned amyloid fibril samples show that the core structure is conserved. Our apparatus and methods have more wide-spread applications in the analysis of fibrous protein structure.

P-725

Dynamics of hemoglobin and water in human red blood cells and concentrated hemoglobin solutions

A. M. Stadler[1], K. Zerlin[2], I. Digel[3], G. Artmann[3], J. P. Embs[4], G. Bueldt[2], G. Zaccai[1]

[1]Institut Laue-Langevin, Grenoble, France, [2]Research Centre Juelich, Juelich, Germany, [3]University of Applied Science, Juelich, Germany, [4]ETH Zurich & Paul Scherrer Institut, Villigen, Switzerland

We studied molecular dynamics of hemoglobin and diffusion of water in whole human red blood cells and concentrated human hemoglobin solutions with incoherent elastic and quasi-elastic neutron scattering. Protein dynamics and water diffusion were measured on time scales of around 1ns, 100ps and 10ps by using several neutron spectrometers with different energy resolutions.

We could show that there occurs a transition of hemoglobin dynamics and diffusion of water at human body temperature of 37°C. The measurements of protein dynamics show that hemoglobin becomes more rigid at temperatures higher than human body temperature. By contrast we found a step-like increase of water diffusion at human body temperature.

We think that hemoglobin molecules begin to form aggregated clusters at human body temperature. The protein intermolecular forces would thus have a stabilizing effect on hemoglobin dynamics. Additionally we consider that water molecules which are tightly bound in the hydration shell of hemoglobin at temperatures lower then body temperature start leaving the hydration shell at human body temperature and become faster moving water. It is important to notice that these transitions occur in whole red blood cells and we believe that these phenomena also occur in the cells in the human body.

O-726

Structural disorder and the functional promiscuity of proteins

P. Tompa

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary

Basic mechanisms by which individual proteins may increase network complexity is the ability to bind multiple partners, and to moonlight, when the same protein fulfils more than one functions. Traditionally, this phenomena are attributed to separate binding surfaces of globular, folded proteins. Here we we suggest that radically different mechanisms may be provided by intrinsically unstructured proteins (IUPs), which present large interaction surfaces for multiple interactions and enable structural adaptability for binding unrelated partners with potentially different effects. For the role of structural disorder in multiple binding, proteins of different connectednesses were compared in the interaction networks of *S. cerevisiae*, *D. melanogaster*, *C. elegans* and *H. sapiens* with respect to the distribution of predicted structural disorder, sequence repeats, low complexity regions and chain length. Highly connected proteins contained significantly more of, and a greater proportion of, these sequence features, and tended to be longer overall as compared to less connected proteins. In terms of multiple functions, we have collected eleven examples, the structural malleability of which gives rise to unprecedented cases of moonlighting, with opposing – inhibitory and activatory – action on different partners or even the same partner molecule. Due to the apparent functional benefits of these parsimonious uses of protein material, we suggest that many more such examples might exist in complex metabolic networks.

P-727

Can the antioxidative effects of green tea catechins inhibit 1800 MHz radiofrequency radiation-induced protein oxidation?

A. Tomruk, E. Ozgur, G. Güler, N. Seyhan

Gazi University Faculty of Medicine/Biophysics Department, Ankara, Turkey

The most popular source that people are exposed to is mobile phones which are source of radiofrequency electromagnetic fields (RF-EMF). Scientists have focused on the possible biological effects of mobile phones which can cause oxidative effect which means that increased damage causing reactive oxygen intermediates, also called "free radicals". Oxidative damage can be depressed by antioxidants such as green tea catechins, (-)-epigallocatechin-gallate (EGCG). In this study, it was aimed to investigate whether the antioxidative effects of green tea catechins can inhibit RF-EMF – induced reactive oxygen species (ROS) release causing oxidative damage of proteins in guinea pigs' liver tissue. RF-EMF generated by mobile phone with 0.81 W/kg digital SAR value operating in GSM 1800 MHz frequency. Male Guinea pigs were exposed to mobile phone radiation averaged as 11.2 V/m, measured during exposure for 20 minutes in 7 days of a week. Guinea pigs divided into four group as controls, EGCG- administrated, mobile phone exposed and mobile phone exposed with EGCG. The oxidative modification of proteins via ROS was investigated for each experiment groups by measurement of the protein carbonyl content (PCO) in liver tissue. As a result, PCO both in the mobile phone exposed and mobile phone exposed with EGCG decreased significantly.

Abstracts

– Disordered and aggregated proteins –

P-728

Trp-Trp energy migration as tool to follow protein unfolding

N. V. Visser[1], A. H. Westphal[2], A. van Hoek[1], C. P. van Mierlo[2], H. van Amerongen[1], A. J. Visser[2]

[1]Laboratory of Biophysics, Wageningen University, P.O. Box 8128, 6700 ET Wageningen, The Netherlands, [2]Laboratory of Biochemistry, Wageningen University, P.O. Box 8128, 6700 ET Wageningen, The Netherlands

The understanding of how a linear chain of amino acids folds into a functional protein molecule with a complex three-dimensional structure is one of the major challenges in structural biology today. Folding of a protein to its native state can go through many distinctive pathways. Here, using apoflavodoxin as a model protein, we follow protein unfolding with polarized fluorescence spectroscopy monitoring the emission of the three tryptophanyl residues at picosecond time resolution. In the native, folded state analysis of the fluorescence anisotropy decay reveals the presence of correlation times on picosecond and nanosecond timescale. We conclude by comparing simulated and experimental results that the main depolarization mechanism is due to homo-transfer of energy between pairs of tryptophan residues. Since the critical transfer distance between two tryptophan residues is rather small, ~ 1.0 nm, any change in distance and orientation during the unfolding process can be immediately measured via changes in fluorescence anisotropy decay parameters.

Abstracts

– Photodynamic therapy –

P-729

Optical pharmacokinetics for photosensitizer chemometrics

M. R. Austwick[1], J. Woodhams[1], C. Elliot-Laize[1], V. Chalau[1], A. J. MacRobert[1], I. J. Bigio[2], S. G. Bown[1]

[1]National Medical Laser Centre, University College London, London, U.K., [2]Boston University, Boston, USA.

Introduction: Measuring the concentration of a photosensitising drug (PS) non-invasively in tissues could provide substantial benefits for photodynamic therapy (PDT). The aim of this study was to assess the potential and limits of using Elastic Scattering Spectroscopy (ESS) for Optical Pharmacokinetics (OP) (measuring the tissue concentration of the PS Aluminium Disulphonated Phthalocyanine (AlS_2Pc) *in vivo)* to see if this helped predict drug dose and the extent of PDT necrosis. **Materials & Methods:** AlS_2Pc was given intravenously to Wistar rats (0.1-5mg/kg), 1-24 hours prior to OP measurements in the liver, colon, skin, muscle, oral mucosa and stomach. For comparison, AlS_2Pc in these tissues was measured using chemical extraction. In a separate group of animals, AlS_2Pc PDT was also performed on the liver and colon (670nm, 50J at 100mW via a bare cleared 400μm fibre) where OP measurements were taken just prior to light delivery in the oral mucosa and the target organ. OP data was correlated with *ex vivo* chemical extraction. **Results & Conclusions:** AlS_2Pc tissue levels were assessed from the OP spectra by analysis of the height of the absorption peak and the area under the absorption curve (650-700nm). All OP results correlated well with chemical extraction. Spectral analysis method was refined to eliminate the need to take a reference measurement on unsensitised tissue. OP is a promising real time method for quantitative pharmacokinetics of PS *in vivo*.

P-730

Targeted photodynamic therapy with multiply loaded recombinant antibody fragments

M. Bhatti[1], G. Yahioglu[2], L. R. Milgrom[2], M. Garcia-Maya[1], K. A. Chester[3], M. P. Deonarain[1]

[1]Division of Cell & Molecular Biology, Faculty of Natural Sciences, Imperial College London, Exibition Road, London SW7 2AZ, U.K., [2]PhotoBiotics Ltd, 21 Wilson Street, London, EC2M 2TD, U.K., [3]Dept. of Oncology, Royal Free and University College Medical School, University College London, Rowland Hill Street, London NW3 2PF, U.K.

Current photodynamic therapy (PDT) of cancer is limited by inefficiences involved in specifically targeting photosensitisers to tumours. Although antibodies are being explored as targeting vehicles, they present significant challenges, particularly in terms of pharmacokinetics and drug-coupling. We describe here a novel and effective system to covalently attach multiple photosensitiser molecules (both pre-clinical, pyropheophorbide-a and clinically approved, verteporfin photosensitisers) to single-chainFvs. Further, we demonstrate that not only do the resulting photoimmunoconjugates retain photophysical functionality, they are more potent than either free photosensitiser, effectively killing tumour cells in vitro and in vivo. For example, treatment of human breast cancer xenografts with a photo-immunoconjugate comprising an anti Her-2 scFv linked to 8 molecules of pyropheophorbide-a leads to complete tumour regression. These results give an insight into the important features that make scFvs good carriers for PDT drugs, and provide proof of concept of our unique approach to targeted photodynamic therapy (tPDT). This promises to significantly improve upon current photodynamic therapy for the treatment of cancer.

P-731

Kinetics of hypericin incorporation into LDL

L. Buriankova[1], V. Huntosova[1], D. Buzova[1], P. Miskovsky[2], D. Jancura[1]

[1]Department of Biophysics, P.J.Safarik University, Kosice, Slovakia, [2]International Laser Center, Bratislava, Slovakia

By means of UV-VIS absorption and fluorescence spectroscopy we have studied the kinetics of the incorporation of hypericin (Hyp), a natural photosensitizing pigment, into low-density lipoproteins (LDL) and transfer of Hyp molecules between LDL particles. Biphasic kinetics of Hyp association with LDL was observed when solutions of Hyp and LDL were mixed together at various concentration ratios. The rapid phase of Hyp incorporation is completed within seconds, while the slow one lasts several tens of minutes. This suggests that the process of Hyp interaction with LDL is nontrivial and the existence of two types of binding sites for Hyp in LDL is proposed. The kinetics of the incorporation of Hyp into LDL particles pre-loaded with Hyp (Hyp/LDL=50:1) was also investigated. The observed decrease of Hyp fluorescence is sign of the formation of aggregates as well as of the dynamic quenching of singlet excitation state of Hyp molecules inside LDL. The characteristic time for this process is comparable with the time of the slow phase of the Hyp incorporation into LDL particles. To study the kinetics of the transfer of Hyp molecules between LDL particles, the time dependence of the fluorescence and absorbance of Hyp was followed after the mixing of the complex Hyp/LDL=200:1 with appropriate amounts of free LDL. For each final Hyp/LDL ratio the increase of the fluorescence and absorbance intensity of Hyp was observed. The half-time of this process is similar to that one of the slow phase of Hyp incorporation into LDL.

O-732

Photothermal sensitisation as a novel therapeutic approach for tumours

G. Jori

Department of Biology, University of Padova, Italy

Photothermal therapy (PTT) is based on the promotion of photothermally sensitised processes in tumour tissues, which reflect the tendency of a chromophore to dissipate electronic excitation energy via a rapid cascade through vibrational modes resulting in increased kinetic energy of the adjacent matrix species. The temperature rise can reach 130 °C above the basal value: water molecules tend to vaporize setting up an acoustic shock wave that propagates through the medium causing mechanical/chemical damage. Using short laser pulses allows the possibility of absorption of other photons in the same volume before the temperature spike has dissipated, promoting an increased shock wave activity. Irradiation of amelanotic melanoma cells with 850 nm light from a Ti:sapphire laser, operated in a pulsed mode at high fluence rates and in the presence of Ni(II)-octabutoxy-naphthalocyanine (NiNc), promoted a photothermally sensitised process leading to fast and irreversible cell death. This resulted in the ejection of a consistent mass of cytoplasmic material from the irradiated cells as detected by scanning electron microscopy. The efficiency of the photoprocess was modulated by intracellular NiNc concentration and the formation of aggregated NiNc clusters in specific subcellular areas, while it appeared to be independent of oxygen. Similar results were obtained upon irradiation of human amelanotic melanoma cells and transformed murine fibroblasts. Thus, photothermal sensitisation appears to be a general phenomenon: studies with melanoma-bearing mice, irradiated at 24 h after NiNc injection, suggest that PTT has potential for the therapy of at least some types of skin tumours.

Abstracts

– Photodynamic therapy –

P-733

Porphyrin oligomers for photodynamic therapy via two-photon excitation

M. K. Kuimova[1], D. Phillips[1], H. Collins[2], M. Balaz[2], E. Dahlstedt[2], H. L. Anderson[2], N. Sergent[3], J. Levitt[3], K. Suhling[3]
[1]Department of Chemistry, Imperial College London, UK, [2]New Chemistry Research Laboratory, University of Oxford, UK, [3]Department of Physics, Kings College London, UK

Photodynamic therapy (PDT) is a minimally invasive procedure for treating of neoplastic diseases.[1] It is based on the light activation of the photosensitizer molecules, which are preferentially localised in the target tissues. Two-photon excited photodynamic therapy (TPE-PDT) utilises chromophores with a high two photon absorption (TPA) cross section as photosensitizers. The TPA means that absorption of light is confined to a very small focal volume of the pulsed laser and in PDT this could be used to reduce the light induced out-of-focus damage to the adjacent healthy tissue. This combined with deeper tissue penetration by red light offers scope for more efficient treatment of age-related macular degeneration.[2]
We propose to use Zn-containing conjugated porphyrin dimers as the efficient photosensitizers with extremely large TPE absorption cross sections.[3] We report our recent progress in the functionalisation of the dimer core to enable efficient delivery into living cells. Confocal fluorescence imaging, fluorescence lifetime imaging and *in vitro* cytotoxicity experiments will be described for a series of lypophylic dimers.
1. R. Bonnett, Chemical Aspects of Photodynamic Therapy, *Gordon and Breach Science Publushers*, Amsterdam, **2000;** 2. J. D. Bhawalkar, et al, *J Clin Laser Med Sur*, **1997**, *15*, 201; 3. M. Drobizev et al, *J. Am. Chem. Soc*., **2004**; *126*, 15352.

O-734

Photosensitisers for photodynamic therapy and photochemical internalisation

S. MacRobert
National Medical Laser Centre, Royal Free and University College Medical School, University College London, London, UK

Many new photosensitisers have been developed in recent years for photodynamic therapy of cancer and non-oncological applications such as bacterial infections. Some of these sensitisers act primarily on tumour cells whereas as others exert their effect through vascular damage. New detection techniques for measuring the generation of singlet oxygen, the cytotoxic species believed to be involved in PDT, have also been reported. These advances will be reviewed together with new related applications. Photochemical internalization (PCI) is a novel technology based on photodynamic therapy, which is being developed for improving delivery of drugs to cells using photosensitised redistribution of the drugs within cells. Many anticancer agents have high inherent toxicity but have limited efficacy in cells since they are taken up via endocytosis and become sequestered within lysosomal vesicles. We have investigated whether we could enhance delivery of chemotherapy drugs to their intracellular target sites, and in turn their therapeutic efficacy, by photochemical internalisation.

P-735

Correlation between LDL-delivery system, subcellular distribution and PDT efficiency of hypericin in U-87MG cells

Z. Nadova[1], V. Huntosova[1], A. Mateasik[2], J. Mikes[3], P. Miskovsky[1]
[1]Department of Biophysics, P.J. Safarik University, Kosice, Slovak Republic, [2]International Laser Centre Bratislava, Slovak Republic, [3]Institute of Biology and Ecology, P.J. Safarik University, Kosice, Slovak Republic

Low-density lipoproteins (LDL) play a key role in the delivery of hydrophobic photosensitizers to tumor cells in photodynamic therapy (PDT). Hypericin (Hyp) is a natural photosensitizing pigment which displays an antiproliferative and cytotoxic effect on tumor cells. The dependence of the uptake of Hyp by human glioma U-87 MG cells on the level of expression of LDL receptors has been studied. The results show that the intracellular concentration of Hyp in U-87 MG cells in the presence of LDL is proportional to the Hyp/LDL molar ratio. A special role of the LDL receptor pathway for Hyp delivery to U-87 MG cells was confirmed by the increase of Hyp uptake and in the situation when number of LDL receptors on the cell surface was elevated. Moreover, the co-localization experiments showed the lysosomal localization of Hyp following the uptake and that the concentration of Hyp in these organelles was enhanced in the cells with elevated number of LDL receptors.
Finally a correlation between PDT effectiveness, Hyp delivery into the cells (different incubation media, activated and non-activated LDL receptors) and Hyp sub-cellular localization has been investigated.
Acknowledgements: This work was supported by the contract APVV-20-036104

O-736

Observing the irradiation of a photosensitizer incorporated in giant unilamellar vesicles

K. A. Riske[1], T. Sudbrack[2], N. L. Archilha[2], A. U. Fernandes[3], M. S. Baptista[3], R. Itri[2]
[1]Biophysics Dept. - Unifesp, Sao Paulo, Brazil, [2]Institute of Physics - USP, Sao Paulo, Brazil, [3]Institute of Chemistry - USP, Sao Paulo, Brazil

The damage inflicted on cell membranes due to irradiation of a photoactive molecule is one of the key steps leading to cell death in photodynamic therapy (PDT). Porphyrines are one of the most used photoactive molecules in PDT. In this work we investigate the effect of irradiating a porphyrine attached to a phospholipid headgroup (PE-porf) incorporated in giant unilamellar vesicles (GUVs) composed of the unsaturated lipid palmitoyl oleoyl phosphatidylcholine (POPC). One of the main targets of the reactions started with the excitation of photoactive molecules is the double-bonds found in the acyl chains of membrane lipids. GUVs containing 2-20 mol% PE-porf were observed by phase contrast optical microscopy. After few seconds of irradiation with focused mercury lamp, initially spherical GUVs gained excess area, started to fluctuate and very often expelled several small buds. The velocity of area increase was correlated to the percentage of PE-porf incorporated, and determined the extent of small buds formation. Geometrical analysis of the deformation of GUVs containing 2 mol% PE-porf (when no evident buds were seen) revealed an area increase of about 2%. Short-chains fatty acids generated from chain breakage at the double-bonds are one of the possible by-products caused by photosensitizer excitation. Thus, the area increase is most probably related to the accommodation of some of these fatty-acids inside the bilayer.

Abstracts

– Photodynamic therapy –

O-737

Biophysical features of the VEP

N. S. Stanciu
Colentina Neurology Hospital & Rom.Soc. of Pure & Applied Biophysics, Univ. of Medicine and Pharmacy, Bucharest, Romania

The objectives of the work:
To show the relationship between the features of the visual evoked potential - amplitude, latency - and the velocity of transmission and the energy included in the signals connected to visual processing.
Methods:
We used an EEG analyzer Zwonitz 2000 and a couple of silver electrodes situated on the occipital areas of the brain with Fz reference. The stimulation was done by white flashes from a distance of approximately 30 cm. Amplitudes and latencies for all the VEP components, (N20, N40, P50, N70, P100, N150, N220) were estimated in 20 normal subjects..
Processing:
Using the average values obtained we performed a numeric estimation of the velocity of signals for each component of the VEP, by dividing the value of estimated visual trajectory length through the latency of the signal. We squared the amplitude values to obtain a numerical estimation of the energy needed for processing. Dividing the two above mentioned value we obtained a coefficient of energy that expresses the efficiency of the visual inflow transmission for a 1m /s velocity.
Results & Conclusions:
We conclude that the brain is optimally processing the visual signals, with a low energy cost, and a very constant velocity. Considering the numerical values we could estimate some clinical dysfunctions of the occipital visual areas and their development or improvement in pathologic subjects.

O-738

Photodynamic therapy (PDT) for treating age related macular degeneration (ARMD)

H. van den Bergh, E. Debefve, J.-P. Ballini
EPFL-ENAC-LPAS, CH-1015 Lausanne, Switzerland

Vascular hyperproliferation characterises several diseases, including cancer and age-related macular degeneration (AMD). One therapeutic approach that is based in part on the closure of the vascular networks associated with these diseases is photodynamic therapy (PDT). The final result is vascular collapse and blood flow stasis leading to the occlusion of the pathological irradiated neovascularisation.
In the treatment of choroidal neovascularisation (CNV) associated with age-related macular degeneration (AMD) two major clinical advances have been demonstrated in the past decade. The first is PDT. The second is anti-vascular endothelial growth factor (anti-VEGF) therapy. PDT closes CNV efficiently and rapidly but also tends to locally enhance the release of angiogenic cytokines like VEGF. This leads to undesired reperfusion and neoangiogenesis, and thus finally the necessity of retreatment. Anti-VEGF therapy is used to treat CNV in AMD and helps to block reperfusion and neoangiogenesis.
We develop a novel combination therapy approach, in which we take advantage of the transient enhanced vascular permeability in PDT, to selectively release at the location where PDT is inducing the vascular response, chemotherapeutic agents in the case of treatment of a malignant tumor or antiangiogenic factors to prevent growth/regrowth of the neovasculature in the case of AMD. The synergy of the highly localised chemotherapy with local closure of the blood vessels that supply the tumour then leads to new and more effective treatment possibilities for various neoplasms.

Abstracts

– Motor proteins in RNA/DNA metabolism –

P-739

Regulation of N-type calcium channel transcript stability by the stargazin-related protein γ_7

L. Ferron[1], D. J. Cox[1], J. Leroy[1], K. M. Page[1], M. Nieto-Rostro[1], S. Bolsover[2], A. J. Butcher[1], A. Davies[1], J. Gruendemann[1], P. Sellaturay[1], W. S. Pratt[1], F. J. Moss[1], A. C. Dolphin[1]

[1]Departments of Pharmacology, University College London, London, U.K., [2]Departments of Physiology, University College London, London, U.K.

The role of the novel putative stargazin-like γ-subunits in relation to voltage-gated calcium channel function is controversial. We have previously cloned a member of this family, γ_7, and found that it markedly reduced functional expression of $Ca_V2.2$ channels. Here we have shown the degradation rate of $Ca_V2.2$ mRNA is increased in the presence of human γ_7 (after 9 hours with Actinomycin D: 45.8 ± 14 %, n=11, *vs* 11.9 ± 3.6 %, n=7, with γ_7 and under control conditions, respectively; mean±SEM, $P<0.05$, Student's *t*-test). This process regulates the physiological level of $Ca_V2.2$ transcripts, since knockdown of endogenous γ_7 with short hairpin RNA constructs in the neuron-like PC12 cell line markedly increased their endogenous $Ca_V2.2$ mRNA level. Interestingly, the knockdown of endogenous γ_7 also affects neurite outgrowth of differentiated PC12 cells and native DRG neurons. We have shown that the reduction of neurite outgrowth is associated with modification of F-actin and GAP-43 distribution in growth cones. Our results support the hypothesis that γ_7 is involved in the stability of specific mRNAs such as $Ca_V2.2$ and that this function is involved in neurite outgrowth.

P-740

Simulation of the Equilibrium Folding/Unfolding of a Small RNA Hairpin

A. E. Garcia[1], D. Paschek[2]

[1]Department of Physics, Applied Physics and Astronomy, Rensselaer Polytechnic Institute, Troy, NY 12180 USA, [2]Physikalische Chemie, Otto-Hahn-Str. 6, Universität Dortmund, D-44221 Dortmund, Germany

We report molecular dynamics simulations of the equilibrium folding/unfolding thermodynamics of the RNA tetraloop in explicit solvent. A replica exchange molecular dynamics study of the r(CGUUGCCG) oligomer that forms a hairpin is performed for 226 ns per replica, using 52 replicas. We are able to show the unbiased folding of all replicas starting from extended conformations. The equilibrium pressure-temperature free energy of folding, $\Delta G(P,T)$, is calculated from the averaged energy, pressure, and specific volume change upon folding of the oligomer as a function of T at constant volume. We find this oligomer is destabilized by increasing hydrostatic pressure, similar to the behavior of globular proteins.

O-741

Single molecule detection of six pRNAs and direct observation of phi29 DNA-packaging motor with customized single molecule dual-view system

P. Guo, W.-D. Moll, D. Shu, H. Zhang

Department of Comparative Pathobiology, Weldon School of Biomedical Engineering, Purdue University, USA

Bacterial virus phi29 DNA packaging motor is geared by the ATP-binding RNA that binds to the connector containing a 3.6-nm central channel as a path for DNA. A switchable mimetic DNA-packaging motor has been constructed. The 3D structures of pRNAs have been probed by photoaffinity crosslinking, chemical modification interference, nuclease probing, cryo-AFM and 3D computer modeling. The active motor was stalled and restarted to observe DNA motion in real time. A single fluorophore dual imaging system was constructed to count the number of fluorescent-pRNA directly within the motor. The stalled motors with partially packaged DNA were identified by co-localization of Cy3 and Cy5, the dual labels to pRNA and DNA respectively. Precise calculation of identical or mixed RNA building blocks of one, two, or three within the biological complexes and six copies within the motor were demonstrated by photobleaching assay and evaluated by binomial distribution. Up to six ferritin or gold particles were observed on the procapsid when each pRNA were conjugated with one particle. The five-pRNA ring was inactive in phi29 DNA packaging. The histograms of photobleaching steps for pRNA were almost identical in procapsid/pRNA complexes and in DNA packaging intermediates, indicating that all six pRNA stayed on the motor during DNA translocation. The imaging system is useful in single molecule FRET or polarization studies. In combination with electromagnetic devices, the motor functions were extensively investigated.

O-742

Single-molecule studies of sigma54-dependent transcription

M. Heilemann[1], K. Lymperopoulos[1], S. Wigneshweraraj[2], M. Buck[2], A. N. Kapanidis[1]

[1]Department of Physics and IRC in Bionanotechnology, University of Oxford, Oxford, United Kingdom, [2]Department of Biological Sciences, Imperial College London, London, United Kingdom.

Transcription, the vital process of copying genetic information from DNA to RNA, is orchestrated by RNA polymerase (RNAP). In bacteria, RNAP directs transcription after forming a functional complex ("holoenzyme") with transcription-initiation proteins known as sigma factors. Most of the published work focuses on sigma70-dependent transcription; we study sigma54-dependent transcription which, in contrast to sigma70-transcription, requires ATP hydrolysis, specific DNA sequences ("enhancers"), and specific activator proteins to form transcription-competent complexes.

Here, we present studies of sigma54-dependent transcription using single-molecule fluorescence resonance energy transfer (smFRET) and alternating-laser excitation (ALEX) spectroscopy. Using site-specifically labelled sigma54 proteins and site-specifically labelled promoter-DNA fragments, we demonstrate that we can observe single diffusing transcription-initiation sigma54-DNA and RNAP-sigma54-DNA complexes, and that we can measure distances and distance changes within such complexes; the identity of the complexes has also been confirmed using electrophoretic-mobility-shift assays. Our studies pave the way for understanding the mechanism of abortive initiation and promoter escape in sigma54-dependent transcription.

Abstracts

– Motor proteins in RNA/DNA metabolism –

O-743

RNA helicases: ATP-driven switches and motors for RNA structural changes

E. Jankowsky
Department of Biochemistry & Center for RNA Molecular Biology, Case Western Reserve University, Cleveland, OH, USA

RNA helicases are the largest group of enzymes in eukaryotic RNA metabolism. Although RNA helicases are structurally highly similar to each other and to DNA helicases, their mechanisms of action are very diverse, and not limited to translocation along one strand of nucleic acids. Here I describe mechanisms for RNA duplex unwinding for two different RNA helicases, the viral helicase NPH-II and the yeast helicase Ded1. NPH-II behaves like a canonical helicase and translocates processively along one RNA strand, thereby removing either complementary nucleic acid or bound proteins. Ded1, which belongs to the DEAD-box protein group, does not translocate. It initiates unwinding directly from duplex regions, including internal positions, and it utilizes single stranded substrate regions to faciliatete loading on the duplex. Actual strand separation occurs through local duplex separation. While the translocation-based mode of duplex unwinding by NPH-II appears well suited for RNA transactions in viral replication, the switch-like mechanism by which Ded1 unwinds RNA duplexes explains many observation with other cellular DEAD-box proteins and may be the prototypical mode by which RNA duplexes are separated in eukaryotic RNA metabolism.

P-744

Non-equilibrium fluctuations and mechanochemical coupling of a molecular motor

D. Lacoste[1], A. W. Lau[3], K. Mallick[2]
[1]Laboratoire PCT, ESPCI, 10 rue Vauquelin, 75231 PARIS Cedex 05, FRANCE, [2]Service Physique Theorique, CEA Saclay, 91191 Gif, FRANCE, [3]Dept. of Physics, Florida Atlantic University, 777 Glade Rd., Boca Raton, FL 33431, USA

We investigate theoretically the violations of Einstein and Onsager relations, and the efficiency of a single processive motor operating far from equilibrium using an extension of the two-state model introduced by Kafri et al. [Biophys. J., **86**, 3373 (2004)]. With the aid of the Fluctuation Theorem, we analyze the general features of these violations and this efficiency and link them to mechanochemical couplings of motors. In particular, an analysis of the experimental data of kinesin using our framework leads to interesting predictions that may serve as a guide to future experiments.

P-745

DNA translocation by RecG helicase

B. Powell, M. R. Webb
MRC National Institute for Medical Research, London, U.K.

RecG is a bacterial, monomeric, superfamily II DNA helicase which plays a role in DNA repair processes through the rescue of stalled replication forks. The preferred substrates of RecG are forked DNA structures to which it binds with high affinity. It is capable of catalyzing migration of these branched structures by an ATP dependent translocation mechanism which is thought to reverse collapsed forks to a position upstream of the lesion to allow repair followed by replication restart.

Previous work in this laboratory has used junctions with heterologous arms. This work characterizes the activity of RecG with fully homologous junctions, which are more representative of the natural substrate. The kinetic mechanism of RecG has been examined using two complementary approaches. DNA translocation rates are measured by observing the separation of a fluorophore/quencher pair during unwinding of substrates created from synthetic, labeled oligonucleotides. These data are combined with analysis of ATP hydrolysis during the process, determined using a phosphate biosensor-based assay, to allow a more complete view of RecG activity.

RecG is found to translocate through 4-strand homologous junctions and hydrolyze ATP at a rate that is independent of substrate arm length. During translocation each arm of the substrate is unwound simultaneously through a coupled process. Heparin can be used to trap RecG molecules that have dissociated from their substrate. Using this technique, premature termination of translocation has been observed during unwinding of longer armed junctions, indicating a low processivity on these DNA structures.

P-746

First steps on the folding pathway of Onconase

C. Schulenburg, U. Arnold, R. Ulbrich-Hofmann
Martin-Luther University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, 06120 Halle, Germany

Onconase, a ribonuclease from the Northern leopard frog *Rana pipiens*, is the smallest member of the ribonuclease A superfamily. Despite the similar tertiary structure, this enzyme differs from the wellknown ribonuclease A by a high cytotoxicity and an unusually high stability. After we have shown by sitedirected mutagenesis that several structural characteristics of Onconase such as the N-terminal pyroglutamate residue, a typical hydrophobic cluster, and an additional disulphide bond in the C-terminal region contribute to its higher stability (*Biochemistry* (2006), 45, 3580-3587), here the folding pathway of Onconase is studied by fluorescence spectroscopy. Manual and stoppedflow mixing experiments (single and double jumps) in 0 – 7 M guanidine hydrochloride allowed to differentiate three folding and unfolding phases, indicating the presence of at least two intermediates on the folding pathway. The folding rate constants differ by one order of magnitude from each other and range from 0.1 to 10 s^{-1}. Binding experiments with the fluorescent dye 8-anilino-1-naphthalene sulfonate reflect the same folding phases as intrinsic fluorescence and show that the intermediates differ in their hydrophobicity. Stopped-flow reactivation studies performed with the fluorescent substrate 6-carboxyfluorescein-dArU$(dA)_2$-6-carboxytetramethylrhodamine indicate that only the native state is active. Although Onconase contains four proline residues, folding steps caused by *cis/trans* isomerisation can be excluded as experiments with the *cis/trans* isomerases Thermus Sly D and Cyclophiline 18 suggest.

Abstracts

– Motor proteins in RNA/DNA metabolism –

O-747

Mechanism, control and diversity of molecular motors on DNA

R. Seidel[1], J. Bloom[3], C. Dekker[3], M. D. Szczelkun[2]
[1]Biotechnological Centre, University of Technology Dresden, Germany, [2]DNA-Protein Interactions Unit, Department of Biochemistry, University of Bristol, U.K., [3]Kavli Institute of Nanoscience, Delft University of Technology, The Netherlands

Many genetic processes require the action of large protein machines that act on DNA as a function of ATP hydrolysis. In this way these complexes act as "molecular motors" – they convert chemical energy as ATP into mechanical events. Our lab is interested in how ATP hydrolysis is used to communicate between distant sites on DNA, sites which may be many thousands of base pairs distant. The Type I and III Restriction Enzymes are complex molecular machines that protect bacteria by cutting invading viral DNA but do so only after interacting with multiple recognition sites in an ATP-dependent manner. The coupling of ATP hydrolysis to cleavage is due to the presence of helicase motifs, although the none of these enzymes unwind DNA. We have recently shown that Type I motors move along intact dsDNA using motor contacts to the 3'-5' strand. We are now developing novel assays to investigate precisely what these motor-nucleases do before cleaving DNA.

O-748

smFRET reveals nucleotide- and RNA-induced conformational changes in RNA-helicase mediated unwinding

B. Theissen, A. R. Karow, D. Klostermeier
University of Basel, Biozentrum, Biophysical Chemistry, Basel, Switzerland

RNA helicases are energy coupling enzymes that convert the energy of ATP hydrolysis into structural changes in their RNA substrates. They mediate a multitude of RNA rearrangements in transcription, splicing, RNA editing, translation, RNA export, ribosome assembly, RNA degradation, and potentially RNA folding in general. DEAD box helicases share conserved motifs, among them the name-giving DEAD box. Structurally, DEAD box helicases consist of a helicase core comprising two RecA-like domains connected by a flexible linker. In some cases, N- or C-terminal regions flanking the core confer substrate specificity or mediate interactions with other proteins. The conserved helicase motifs line a cleft between the two core domains, and an opening and closing of this cleft during the helicase cycle has been proposed. Here we present evidence for such a conformational change from single molecule FRET experiments with a double-labeled helicase carrying a donor and acceptor fluorophor on both sides of the inter-domain cleft. While ATP binding does not induce any detectable conformational changes, a species with high FRET efficiency emerges in the presence of RNA, and is more populated in the presence of both ATP and RNA, pointing towards a closure of the inter-domain cleft at the beginning of the unwinding reaction. These results open up avenues to follow ATP-induced conformational changes in the catalytic cycle of RNA helicases in real-time and to understand the role of these movements in RNA unwinding.

P-749

Elucidation of the ATPase cycle for the helicase RecG

C. P. Toseland, M. R. Webb
MRC National Institute for Medical Research, Mill Hill, London, U.K.

The monomeric bacterial helicase RecG brings about replication fork reversal through the atypical translocation of double stranded DNA using ATP. Fork reversal follows replication stalling which occurs when the replication complex reaches a single strand lesion in the DNA. The reversal allows repair of the lesion and continued replication. A translocation step size of 2-3 bp per ATP has been observed for RecG by determination of the translocation kinetics with oligonucleotide junctions. Many other helicases have discrete steps of one base per ATP. For RecG, it has been proposed that a large conformational change leads to the greater step size, but this may possibly consist of smaller sub-steps, brought about through individual steps in the ATPase cycle. This has similarities to some myosin motors and their movement in sub-steps along actin.
In order to elucidate the mechanism by which this chemomechanical coupling occurs, a variety of biophysical techniques have been applied to determine the ATPase mechanism and the individual rate constants. This includes utilising fluorescent analogues of ATP, mantATP/ADP (2'(3')-*O*-*N*-methylanthraniloyl ATP/ADP), to monitor fluorescence intensity in rapid-reaction kinetic experiments, allowing the initial binding and release kinetics to be explored. These analogues are further used for analysis of the hydrolysis step using quenched-flow measurements. Additionally, the fluorescent phosphate binding protein (MDCC-PBP) independently measures the Pi release step. Oxygen exchange experiments using ^{18}O-substituted ATP allow the extent of reversal of the hydrolytic cleavage step and Pi binding to be determined.

O-750

Mechanism of RNA translocation by a viral packaging motor

R. Tuma[1], E. J. Mancini[2], D. H. Bamford[1], J. M. Grimes[2], D. I. Stuart[2]
[1]Institute of Biotechnology, University of Helsinki, Finland, [2]Division of Structural Biology, The Henry Wellcome Building for Genomic Medicine, Oxford University, Oxford, U.K.

The bacteriophages of *Cystoviridae* family package their single stranded RNA genomic precursors into empty capsids using a hexameric packaging motor P4. P4 is an ATPase with similarity to other RecA-like hexameric helicases. Concerted structural, biochemical and mutational studies revealed the mechano-chemical coupling principles. P4 hexamer exhibits sequential cooperativity scheme in which mechanical motion in one subunit enables hydrolysis in the neighboring active site. The functions of several conserved helicase residues in this scheme were delineated in atomic details and compared with those of other hexameric molecular motors. We have also developed a single molecule assay (tethered particle motion) that monitors RNA binding and translocation by P4 hexamer. In the absence of hydrolysable ATP the P4 ring freely diffused along the topologically enclosed RNA strand. The diffusion coefficient depended strongly on the nucleotide binding state and was related to the RNA affinity. In the presence of millimolar ATP P4 had the highest affinity (the lowest diffusion coefficient) for RNA and translocated along the bound RNA in the 5' to 3' direction. At submillimolar ATP P4 often paused and then diffused along RNA with a diffusion coefficient similar to that of the apo-protein. A model of RNA translocation consistent with the structural as well as single molecule observations will be presented.