Oral Presentations
- Lipid protein interactions -

O1-1

Amphipols : polymeric surfactants for membrane biology research

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Membrane proteins classically are handled in aqueous solutions as complexes with detergents. The dissociating character of detergents, combined with the need to maintain an excess of them, frequently results in more or less rapid inactivation of the protein under study. Over the past few years, we have endeavored to develop a novel family of surfactants, dubbed 'amphipols' (APs). APs are amphiphilic polymers that bind to the transmembrane surface of the protein in a non-covalent but, in the absence of a competing surfactant, quasi-irreversible manner. Membrane proteins complexed by APs are in their native state, stable, and they remain water-soluble in the absence of detergent or free APs. An update will be presented of the current knowledge about these compounds and their demonstrated or putative uses at the frontiers of membrane biology.

References

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O1-2

Biophysical approaches to characterize the lateral distribution of proteins and lipids in model membranes

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Using designed transmembrane peptides and mixtures of either synthetic or isolated natural lipids, we are trying to characterize the factors that are involved in determining the lateral distribution of proteins and lipids in membranes. In particular, we focus on (i) the role of hydrophobic matching between the transmembrane segments of proteins with the hydrophobic bilayer thickness, and (ii) the role of specific interactions between cholesterol and phospholipids to form liquid-ordered domains. The systems are characterized by a range of biophysical techniques, including atomic force microscopy, ^{2}H NMR spectroscopy, mass spectrometry and (photo)crosslinking. The results show that a tendency to compensate for hydrophobic mismatch situations can be an important director of membrane organization. However, in systems with different length lipids this does not automatically result in preferential surrounding of peptides with best matching lipids, a situation that could lead to molecular sorting. Also, peptide partioning into liquid ordered phases does not appear to be favourable, even when this would yield the best matching situation. This is most likely due to the tight packing of the lipids in the liquid-ordered phase. In gel phase bilayers, peptides tend to segregate, together with lipids, to form highly ordered striated domains.

O1-3

Cationic peptide interaction with two-component membranes. Domain formation or vesicle aggregation?

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The purpose of the present work was to study the ability of a cationic (+6) model peptide (K6W) to induce the formation of nano-scale domains (or eventually large scale phase separation), in a two-component lipid system containing a neutral phospholipid, DPPC, and a negative one, DPPS. FRET methodologies were used, and the experiment was designed involving derivatized donor and acceptor PC lipids. Because both phospholipid probes have a stronger preference for the fluid phase, if peptide addition to the liposomes induces a lateral segregation of the anionic phospholipids, the surface concentration of acceptors (in the remaining fluid phase) must increase, and the efficiency of energy transfer should increase concomitantly. From steady-state data it was concluded that in the gel-fluid co-existence region, there was no significant variation on FRET efficiency, E, upon the addition of peptide. At variance in the fluid phase, E increased suggesting induced domain formation. However this was ruled out on the basis of time-resolved data analysis, which could only be rationalized on the basis of a model assuming FRET between lipid multilayers. From this work is concluded that lipid molecules are randomly distributed in the fluid phase, and the peptide induces multilayer formation, which could be attributed to vesicle aggregation. There is no evidence for large domain formation.This work also shows that model fitting to FRET time-resolved data, is essential to obtain topological information of membrane model systems, and erroneous conclusions can be derived from steady-state intensity measurements alone.

O1-4

Interactions of antimicrobial peptides with model biomembranes

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We have compared the lipid-protein interactions of several antimicrobial peptides, viz. magainin 2, indolicidin, and temporins B and L using Langmuir films, large unilamellar vesicles, and giant liposomes. All these peptides are highly surface active, penetrating efficiently into phospholipid monolayers, in particular when the acidic phosphatidylglycerol was present. The latter lipid seems to promote its co-segregation with AMPs from phosphatidylcholine, the insertion of the peptide into the bilayer, and increase in lipid acyl chain order. Topical consequences of the peptides were explored using giant liposomes. Pronounced aggregation of the peptides was evident, with subsequent transfer of the aggregated lipid-protein complexes into the internal cavity of the vesicle. We have postulated that it could be the processes following the exposure of the target cell to AMPs which would kill it. In other words, the processes involved in the settling of the system to thermodynamic equilibrium after adding AMPs to the membranes would be bactericidal. Yet, also other factors need to be considered. This is demonstrated by the finding that all four AMPs activate secretory phospholipases A_2, enzymes which could further enhance the damage of the bacterial membranes and their associated functions.

Oral Presentations

- Lipid protein interactions -

O1-5

Membrane fusion and gp41. How HIV enters the cell

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The human immunodeficiency virus (HIV) envelope glycoprotein is composed of a complex between the surface subunit gp120, which binds to cellular receptors, and the transmembrane subunit gp41. Upon activation of the envelope glycoprotein by cellular receptors, gp41 undergoes a sequence of conformationals changes leading to the formation of a fusogenic trimer-of-hairpins that mediate fusion of the viral and cellular membranes, allowing the entry of the virus into the host cell. With a view towards new insights into viral fusion mechanisms, we have investigated by different methodologies, namely, infrared, fluorescence, nuclear magnetic resonance and calorimetry different fragments corresponding to the immunodominant region of the gp41 ectodomain, a highly conserved sequence and major epitope. Information on the structure of the peptide both in solution and in the presence of model membranes, its incorporation and location in the phospholipid bilayer, and the modulation of the phase behaviour of the membrane has been gathered and shows that peptides derived from this gp41 region bind and interact with negatively-charged phospholipids, their conformation changing in the presence of different membranous media, and significantly, these peptides can induce leakage of vesicle contents as well as induce the formation of new phospholipid phases. These characteristics might be important for the formation of the fusion-active gp41 core structure, which may promote the close apposition of the viral and target-cell membranes, providing a plausible mechanism by which membrane fusion may take place.

Oral Presentations
- Theoretical biophysics -

O2-1

Methods for the prediction (and extraction) of protein interactions

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Current High Throughput experimental techniques are providing increasingly complex data in areas such as: genome sequences, expression arrays, yeast two-hybrid (y2h), TAPs/MS and peptide libraries. Bioinformatics and Computational Biology play a crucial role in the organization and analysis of this information. During this presentation I_ll describe the current approaches for the study of protein interaction networks that combine experimental and computational approaches. Regarding the computational methods for the prediction of interaction partners based on sequence information, I will particularly focus on the two methods that my group has recently developed, and the comparison with other computational and experimental approaches (see Pazos & Valencia, Curr. Opinions in Struc. Biol., 2002). I will also review the possibilities open in this field by the application of information extraction technology for the extraction of information from large textual repositories of scientific publications. (for a review see, Blaschke, Hirschman, Valencia, Brief. in Bioinfor., 2002 and Blaschke, Valencia, EEEI, 2002), and how this information can be used for the evaluation of the reliability of the predicted interaction networks. Finally, I will present our view on how the methods developed for the description of protein interaction networks can be tailored for the prediction of the molecular features of individual interactions (protein docking), and protein functional sites (del Sol et al., J.Mol.Biol 2003).

O2-2

The physics of cell locomotion

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The locomotion of a biological cell is based on signal-mediated polymerization of its cytoskeleton. Theoretical considerations and computer simulations have shown that the persistency of the random motion and the chemotaxis of a cell is basically due to the autocatalytic polymerization kinetics of the cytoskeletal actin networks. We discuss the basic conditions under which the motion is performed, as there are substrate coupling, energy supply and network topology.

O2-3

Structure, molecular interactions and dynamics of DNA and RNA. Modern computational view

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Recent developments in computational chemistry led to extensive applications of modern computational methods to DNA and RNA. Ab initio quantum chemical (QM) techniques characterized H-bonding and cation binding of nucleic acid bases, revealed the nature of base stacking and led to the discovery of intrinsic nonplanarity of DNA base amino groups that allows formation of out of plane interactions of bases. QM calculations provide current reference values for the parametrization of other computational tools including modeling force fields. Our latest investigations provide data with close to ultimate accuracy for any kind of nucleobase interactions. A further significant methodological achievement is the advance of explicit-solvent nanosecond scale molecular dynamics (MD) simulations of DNA and RNA. We have used this technique to study molecular interactions in a wide range of important systems, such as DNA qudruplexes, RNA pseudoknots, extended non-Watson-Crick RNA motifs and DNA-drug complexes. Selected examples will be given. The combination of state of the art QM and MD methods provides integrated insights into the role of nucleobase interactions in the structure and dynamics of DNA and RNA. References: P. Hobza, J. Sponer: Chem. Rev. 99, 1999, 3247. P. Hobza, J. Sponer, J. Am. Chem. Soc. 124, 2002, 11802, K. Csaszar, N. Spackova, R. Stefl, J. Sponer J. Mol. Biol. 313, 2001, 1073. N. Spackova, I. Berger, J. Sponer, J. Am. Chem. Soc. 123, 2001, 3295.

O2-4

Determination of the structures of partially folded states of proteins from NMR data

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A combination of computational and experimental methods is making it possible to describe protein folding pathways at atomic resolution. Much of the recent progress has been driven by advances in experimental techniques that provide residue-specific information about the successive intermediate states that are populated during the folding process. Detailed molecular dynamics simulations have been used to complement the experimental results and to suggest new measurements. In an alternative approach discussed here, experimental data are used directly to build an artificial energy function that guides computer simulations to sample the regions of the conformational space of a protein most compatible with the experimental results. In one recent application, NMR data obtained at increasing concentrations of denaturant were used for the determination of the free energy landscape of the molten globule state at pH 2 of alpha-lactalbumin. The resulting landscape is characterized by deep valleys that are robust against changes in the external conditions. These deep valleys in the landscape, created by molecular evolution, define the pathways of folding and ensure the avoidance of misfolded conformations. In another application, results from protein engineering experiments are used to determine the transition state for folding for several proteins. An analysis of the resulting structures suggests that, in the nucleation-condensation mechanism for folding, the transition state is reached when the native-like interactions of few key residues are formed. Taken together, these results illustrate how a combination of computational and experimental data allows the investigation of the general principles of macromolecular self-organization.

Oral Presentations

- Theoretical Biophysics -

O2-5

Characterization of the dizinc analogue of the synthetic diiron protein df1 using ab initio and hybrid quantum/classical molecular dynamics simulation

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A biomimetic four-helix bundle with a binuclear active site (Due Ferro 1, DF1) has been synthesized and characterized. The carboxylate bridged binuclear motif residing in the middle of the four helices resembles the active site of numerous binuclear containing enzymes, such as Manganese Catalase, Methane Monooxygenase, etc. Due to the crucial chemical and biological relevance of binuclear enzymes in hydrolytic as well as redox active processes, we have performed a systematic study of structural and dynamical properties of the dizinc analogue of DF1 through ab initio and hybrid QM/MM (Car-Parrinello) Molecular Dynamics Simulations. Four quantum mechanical representations of the active site have been employed in order to systematically assess the role of first and second shell interactions. In addition, two QM/MM partitioning schemes have been explored in order to explicitly consider the role of the whole protein environment. In the hybrid models, the two transition metals and the coordinated ligands have been treated at first-principles level, while the remaining of the four-helix bundle and the solvent are treated at the molecular mechanical level. All of the calculations confirm the highly flexible nature of the carboxylate-bridged binuclear motif and demonstrate the importance of the whole protein environment in stabilizing the hydrogen bond networks that surround the active site. The QM/MM approach allows for the identification of key factors governing the stability/reactivity of the active site and thus provides unique insights that can be exploited for the future tailoring of new highly selective biomimetic enzymatic compounds.

Oral Presentations

- Emerging techniques -

O3-1

Study by PMIRRAS and Brewster angle microscopy of phospholipid bilayer stabilised at the air/water Interface

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Monolayers at the air/water interface are currently used to study the interactions of phospholipids with peptides or proteins because the physico-chemicals parameters (temperature, surface pressure, salt concentration, pH) of these systems are very easy to control. However, in many cases, the results obtained in these conditions can not be extrapolated to the natural membrane. Indeed, natural membranes are quasi-symmetric and their hydrophobic domain is twice larger than a monolayer. This parameter is crucial because the length of many membrane peptides and proteins often match the thickness of a membrane. The purpose of our work is to stabilise phospholipid bilayers at the air/water interface in view to have a realistic membrane model that can be easily analysed by optical techniques such as PMIRRAS and Brewster Angle Microscopy. In this communication we will present two original methods to realise phospholipid bilayers at the water surface. The first one is derived from the Langmuir technique. A Langmuir trough has been modified to have access to the high surface pressure domain of the isotherm. Under particular conditions of temperature and humidity, several phospholipids can arrange in bilayers at the interface. The second method is analogous to the formation of a supported phospholipid bilayer (SPB) by fusion of small unilamellar vesicules (SUV) on a silicon dioxyde substrate. In a first step, a polymerised glass-like monolayer is formed at the air/water interface. In a second step, SUV are introduced in the subphase and a SPB forms under the monolayer. For these two methods of preparation, PMIRRAS and Brewster Angle Microscopy confirm the presence of a homogenous bilayer at the air/water interface. Moreover, we find that molecular orientations in the bilayer and in the monolayer are similar. Finally we have studied these systems in interaction with melittin, a well-known cytotoxic peptide.

O3-2

Confocal and two-photon excitation techniques from microscopy down to the single molecule level

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The application of confocal and two-photon excitation (TPE) techniques to fluorescence optical microscopy has led to terrific advances in the study of biological systems from the three-dimensional (3D) micro-spectroscopic level down to single molecule detection (SMD) schemes (1, 2). Both techniques are particularly relevant for the study of the 3D and dynamic properties of biological molecules within their natural environment, cells or tissues. In particular the advent of TPE mitigates overall photobleaching and phototoxicity problems, opening new perspectives by providing further attractive advantages (3). Optical schemes and architectures for confocal and two-photon excitation from microscopic level to SMD will be discussed. Moreover, we will address examples of three-dimensional and multiple fluorescence imaging from cells as well as from subresolution objects (4) and single fluorescent molecules (2,5). Emphasis will be given to the utilization of the two-photon microscope as an active device in micro patterning and nanosurgery applications. Recent results in the utilization of confocal and TPE for specific GFP switching at single molecule level and for monitoring of TPE uncaging will be shown. References 1. Diaspro A. (ed.) (2002) Confocal and two-photon microscopy. Wiley-Liss, New York. 2. Chirico G, Cannone F, Beretta, Baldini G, Diaspro A (2001) Micr. Res. Tech. 55, 359. 3. Periasamy, A. (ed.) (2001) Methods in Cellular Imaging, Oxford Univ. Press, N.Y. 4. Diaspro, A. S. Annunziata et al., (2000) Microsc. Res.Tech, 51(5), 464-468. 5. Chirico G., Cannone F., Baldini G., and Diaspro A. (2003) Biophysical J., 84(1), 588-598.

O3-3

Lung fucntion studies by mri with hyperpolarized He-3

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Due to its limited accessibility and intrinsic complex structure, lung is one of the most difficult organs to study non-invasively. Over the past century, only measurements of inspired/expired airflow and pressure have been routinely used for pulmonary function studies. Imaging techniques with radioactive gases allow reliable measurement of regional pulmonary ventilation and perfusion distribution. Despite its better spatial and temporal resolution, conventional Magnetic Resonance Imaging (MRI) techniques had not been currently used with this aim, mainly due to the low proton pulmonary density. However, MRI with hyperpolarized noble gases is very recently been used to obtain new image-derivate pulmonary functional parameters. Compared to proton MRI, noble gases (Xe-129 and He-3) hyperpolarization permits a theoretical 10^5-fold signal-to-noise ratio enhancement. The technique open different possibilities, such as static (ventilation) imaging, dynamic serial imaging, diffusion weighting imaging, and intrapulmonary oxygen concentration, providing a novel approach to simultaneously assess lung function on a topographical basis, and morphology. In this communication, the feasibility of this emerging technique for in vivo analysis of lung functionality, both in human and animal model-based studies, will be firstly examined. Secondly, some results of the apparent diffusion coefficient (ADC) of He-3 obtained by MRI for each pixel of lungs in a rat model of mild emphysema will be presented, showing that these ADC values are proportional to the alveolar sizes, allowing to characterize this disease at earlier stages. Funding: EC: PHIL-QLG1-2000-01559 and MCYT: SAF2000-0115.

O3-4

Micro- and nanolithographical tools for control of integrin clustering and models of the actin cortex

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The lack of higher resolution bio-patterning methods has prevented direct examination of spatial size ranges that contribute to the process of adhesion of a cell on a molecular scale. We designed a hexagonally-close packed template for cyclic RGDfK peptides by self-assembly of diblock copolymers. Nanometer sized adhesive dots are positioned with high precision at 28, 58, 78, and 85 nm spacing in hexagonally-close packed pattern. If adhesive dots are separated by more than 78 nm cell adhesion is constricted. We attributed this cellular response to restricted avb3-integrin clustering. In a second investigation we present a method enabling the self-assembly of freely suspended quasi two-dimensional actin networks by arrays of microscopic pillars that mimick biophysical, biochemical, and structural properties of the intracellular actin cortex of cells, and to quantify its micro-mechanical properties. F-actin can be selectively polymerised from the tops of pillars with the growing ends freely dangling. Addition of the crosslinker filamin induces the formation of suspended orthogonal networks of actin bundles with structural similarity to the actin cortex in cells. The present strategy opens unique possibilities to quantitatively measure physical and structural properties of freely suspended actin filaments under controlled boundary conditions by flicker spectroscopy, actin bundle formation, and to study the transport behavior of processive motor proteins (myosin-V) on pending networks.

Oral Presentations

- Emerging techniques -

O3-5

Combination of modern microscopic techniques (CLSM, AFM, SNOM, FRET) to reveal organization of membrane proteins

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The interaction of cell surface proteins plays an important role in the process of transmembrane signaling. Receptor clustering and changes in their conformation are often essential factors in the final outcome of ligand receptor interactions. Fluorescence resonance energy transfer (FRET) is an excellent tool for determining small scale (1-10 nm) association pattern of cell surface molecules. Applied in confocal laser scanning microscope (CLSM), FRET is a very selective an sensitive tool for resolving spatial heterogeneity of molecular interaction with single cells with a spatial resolution imposed by the inherent diffraction limit (250-400 nm) of optical microscopy. To overcome this limit scanning near-field optical microscopy (SNOM) can be applied for studying the large scale (100-1000 nm) association pattern of membrane proteins. The newly developed technique of SNOM is not limited by diffraction optics, and one can readily image objects in the 100-1000 nm range. In case of FRET, CLSM and SNOM fluorescence labeling provides excellent specificity in biological systems. With atomic force microcopy we can increase the resolution to atomic level, and the dynamic range of the technique can be really wide (1-400 nm). However, the specificity of labeling is lacking, instead of fluorescence labeling gold particles should be applied. Combination of these techniques provides new insights, complementary to each other, about the small and large scale association patterns of cell surface proteins. As examples, cell surface organization of major histocompatibility (MHC) molecules and that of receptor tyrosine kinases will be discussed.

Oral Presentations

- Charge transfer and bioenergetics -

O4-1

Electron transfer from cytochrome bf to photosystem i as mediated by cytochrome c6 and plastocyanin

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O4-2

EPR spectroscopical characterization of the first electron ecceptor in cytochrome c oxidase and ubiquinol oxidase complexes

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O4-3

The thioether bonds in c-type cytochromes-formation and purpose present many puzzles

S. Ferguson
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O4-4

Mitochondrial permeability transition and NAD(P)H oxidation

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Mitochondrial permeability transition and NAD(P)H oxidation

Oxidation of mitochondrial NAD(P)H, induced by various factors, was considered in the literature to be one of the causes, as well as a consequence of the inner membrane permeability transition. In this work, we have observed a biphasic oxidation of the matrix NAD(P)H of rat liver mitochondria in the presence of tert-butylhydroperoxide. After the second phase, mitochondria were uncoupled, while before that the rate of the online ATP synthesis was almost equal to the control value. The second phase may be prevented by cyclosporine A, EGTA or essentially delayed by various antioxidants. The inner membrane potential collapse, permeability transition and mitochondrial swelling predestinated the second phase of rotenone-insensitive oxidation of mitochondrial NAD(P)H and switched in the oxidation of exogenous NADH. Both of these oxidations were explained as a result of the outer membrane rupture and the electron transport from the released or added NADH to the inner membrane cytochrome c oxidase, through flavoprotein Fp5 and cytochrome b5 of the outer membrane and cytochrome c-dependent electron shuttling between the outer and the inner membranes Lemeshko, Biochem. Biophys. Res. Commun. 291, 2002, 170-175; Lemeshko, J. Biol. Chem. 277, 2002, 17751-17757). This work was supported by Grant 1118-05-261-97 of Colciencias.

Oral Presentations

-Charge transfer and bioenergetics-

O4-5

Carrier-like transport kinetics of uncoupling proteins: analysis of planar bilayers reconstituted with UCP1

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Uncoupling protein 1 (UCP1) is known to uncouple substrate oxidation from mitochondrial ATP production and catalyze thereby energy loss as heat. According to alternative hypotheses, UCP1 transports either protons or fatty acid anions. The proton transport rate may serve as a criterion to distinguish between channel and cycling mechanisms. However, turnover rates observed for UCP1 in reconstituted liposomes differ by several orders of magnitude. To solve the controversy, we have used a well-defined system that allows current measurements across planar bilayers reconstituted with UCP1. We found that these bilayers exhibit an increase in membrane conductivity only in the presence of fatty acids. The augmented conductivity was nearly completely blocked by ATP. Direct application of transmembrane voltage and precise current measurements allowed determination of substrate turnover numbers per UCP1 molecule. The maximal rate of 25 per s suggests that UCP1 does not conduct protons via a transmembrane pore. In contrast, the modest turnover number agrees well with the Fatty Acid Cycling hypothesis, according to which UCP1 facilitates the backward transport of fatty acid anions after forward proton transport by flipping protonated fatty acids.

O4-6

Unique or multiple QB binding site in the reaction centers from Rhodobacter sphaeroides

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In the X-ray structures of native reaction center (RCs) from the photosynthetic bacterium Rhodobacter sphaeroides, two distinct main binding sites (distal and proximal) for the secondary quinone QB have been described. The movement of QB from its distal to its proximal position has been proposed to account for the conformational gating step of the first electron transfer from the primary quinone QA- to QB. We have refined the X-ray structures of Pro-L209 mutant RCs in the neutral state [Kuglstatter et al. (2001) Biochemistry 40, 4253]. Whereas the overall protein structures in the mutants are rather similar, QB is observed in different positions. Remarkably, in the mutant RC where Pro-L209 was changed to Tyr, QB is found to occupy exclusively the proximal position. This finding indicates that the position of QB does not solely depend on its oxidation state. In order to test the structural and functional implications of the distal/proximal sites, a comparison of the FTIR vibrational properties of QB in native RCs and in the mutants was performed. Surprisingly, in all RCs, highly specific IR fingerprints of the bonding interaction of QB with the protein are very similar [Breton et al. (2002) Biochemistry 41, 43, 12921]. These FTIR results show that QB occupies the same binding site in all RCs which is favored to be the proximal site. The comparison of the X-ray and the FTIR data with regard to the significance of a functional QB position for the conformational gate of the first electron transfer will be discussed.

Oral Presentations
- Nucleic acid and ribosomes-

O5-1

Structural-Dynamic studies of the RNA Helicase DbpA at atomic and molecular levels

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Protein-RNA interactions play an important role in many biological functions including RNA processing, ribosome assembly, and chaperoning. We are studying the structure-function relationships of the intrigue family of RNA helicases. These enzymes are proposed to be RNA chaperons based on their ability to unwind duplex RNA regions and destabilize protein-RNA complexes. Specifically, we study the dynamic structure-function relationships of the Eschericia coli enzyme, DbpA, which belong to the DEAD (Asp, Glu, Ala, Asp) box RNA helicases family. DbpA is unique in its subclass because it possesses maximal ATPase activity and hence specificity toward the peptidyl transferase center in 23S ribosomal RNA. Therefore, it may play a key role in ribosome biogenesis. Previous studies attempt at the elucidation of the reaction mechanisms associated with both the helicase (unwinding) and ATPase activities of DbpA. Yet, the molecular basis by which these activities are executed by DbpA as well as other RNA helicases remained to be determined. Specifically, for DbpA, the correlation between its specific ATPase activity towards 23S ribosomal RNA and its unwinding activity is not clear. In order to provide new insights to these questions, we conducted dynamic structure-function mechanistic studies on DbpA by introducing new experimental strategies to probe the structural modifications of both the enzyme and its substrates. Our results indicate that the helicase activity of DbpA may be regulated by the structure of its substrate. This may provide new insights to the in vivo biological role of this enzyme.

O5-2

Thermodynamic and Structural aspects of DNA repair in Bacteria

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The bacterium *Deinococcus radiodurans* is the most radiation-resistant organism yet discovered, capable of withstanding ionizing irradiation at doses orders of magnitude higher than any other living system. This tolerance necessitates the reassembly of intact chromosomes from hundreds of DNA fragments generated by radiation, desiccation or other assaults. How *D. radiodurans* restores the integrity of its shattered chromosomes is enigmatic, because the complement of DNA repair enzymes of this organism is strikingly similar to that of other, non-resistant bacteria. We have shown that the ability of the organism to repair multiple DNA breaks derives from the unique structure of its genome. The biophysical and structural aspects of DNA repair processes occurring in bacteria will be discussed. References 1. S. Levin-Zaidman, D. Frenkiel-Krispin, E. Shimoni, I. Sabanay, S. Wolf and A. Minsky. Ordered Intracellular RecA-DNA Assemblies: Site of In-vivo RecA-Mediated Activities. *Proc. Natl. Acad. Sci. USA* **97**, 6791-6796 (2000). 2. S. L. Zaidman, J. Englander, E. Shimoni, A. Sharma, K. Minton and A. Minsky Ring-like Structure of *D. radiodurans* Genome: The Key to Radioresistance? *Science* **299**, 254-256 (2003).

O5-3

Structural basis of stringent response in bacteria

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Oral Presentations

- Protein structure, dynamics and functions (I)-

O6-1

Ferritins, bacterioferritins: iron uptake and storage. Structure of the d. desulfuricans bacterioferritin

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Ferritins constitute a broad superfamily of iron storage proteins, widespread in all domains of life, in aerobic or anaerobic organisms. Ferritins isolated from bacteria, may be haem-free or contain a haem. In the latter case they are called bacterioferritins. The primary function of ferritins inside cells is to store iron in the ferric form. A secondary function may be detoxification of iron or protection against O2 and its radical products. Indeed for bacterioferritins this is likely to be their primary function. Ferritins and bacteroferritins have essentially the same architecture, assembling in a 24-mer cluster to form a hollow, roughly spherical construction. The three-dimensional structure of a bacterioferritin from the anaerobic bacterium Desulfovibrio (D.) desulfuricans was determined in three distinct catalytic/redox states by X-ray crystallography(1). This is the first crystal structure where a native di-iron center in an iron-storage protein has been reported. Furthermore, it has the unique property of having Fe-coproporphyrin III as its haem cofactor(2). Conformational changes support a route for iron entry into the protein shell through a pore that passes through the di-iron center. Molecular surface and electrostatic potential calculations further suggest the presence of another ion channel hypothesized as points of entry for the iron atoms. Electrostatic calculations on other bacterioferritins indicate similar channels. In ferritins, however, this entry route seems is associated with the 3-fold axis channels. These ion channels can be assumed as an iron entry route in the later mineralization processes of core formation. 1- Macedo S. et al. Nature Struct. Biol., in press. 2- Romao, C. V. et al. FEBS Lett. 480, 213-216 (2000).

O6-2

The Catalase-Peroxidase KatG Structure: Activation of Isioniazide and Resilient Mechanisms in Tuberculosis.

Fita, I.
Barcelona, Spain

O6-3

Inhibition of lysosomal cysteine proteases (cathepsins)

Turk D.
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O6-4

Physical aspects of protein dynamics and function

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The architecture of a protein is an important pre-requisite for the understanding of its function. However, it is not sufficient. Equilibrium fluctuations within a conformation as well as dynamical transitions between conformations are essential for the biological function in many cases. For a physical understanding, myoglobin serves as a model system. Many features can be generalised and are valid also for other proteins.

A "Normal Mode Refinement" of X-ray structures of myoglobin in the temperature range from 40K to 300K was used to determine the structural inhomogeneity measured by the mean square displacements, $\langle x^2 \rangle$, of the individual non-hydrogen atoms. A neutron structure analysis with 1.5Å resolution yielded the mean square displacements of the hydrogen atoms at room temperature. It is possible to separate 3 types of hydrogen displacements: backbone like, methyl like and lysine like. A normal mode analysis has given insight into the intramolecular vibrations. Mössbauer effect with synchrotron radiation was used to determine the density spectrum of phonons coupling to the heme iron in myoglobin. From this spectrum the mean square displacements at the position of the iron were calculated and compared with results from Mössbauer absorption spectroscopy.

Conformational changes connected with ligand binding occur only above the "dynamical transition temperature" , T_c; the molecules become biologically active. This is shown by X-ray structure analysis at different temperatures and the relaxation of light induced intermediate states. Mössbauer absorption spectroscopy and incoherent neutron scattering reveal quasi-diffusive intramolecular fluctuations of the backbone and the side chain atoms, respectively, which are the lubricant for conformational changes.

Oral Presentations

- Protein structure, dynamics and functions (II)-

O7-1

Structural genomics: An overview and the Protein Structure Factory

Heinemann, U.
Berlin, Germany.

O7-2

Yeast Structural genomics: High throughput production and crystallization of large sets of eukaryotic proteins.

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South Paris Yeast structural genomics consortium web site: http://genomics.eu.org/ Our structural genomics project aims at the systematic expression, purification and structure determination of yeast proteins of unknown structure. We have chosen 250 targets smaller than 500 amino acids, excluding membrane and coiled coil proteins. We will present our strategy for the systematic cloning, expressing, purification and crystallization. The main difficulties encountered in this pilot project are : (1) the low solubility of a great number of target proteins and (2) difficulties in the crystal optimisation process. We developed parallel strategies to recover proteins from inclusion bodies, including refolding, coexpression with chaperones and in vitro expression techniques. Our project is also involved in robot development for the automatic visualisation of crystallization experiments. A small proto-type robot was constructed representing a capacity to store and analyse about 300 000 crystallization drops. Software is developed to handle the information flow and to spot successful drops in a automatic way. This software is part of our effort to build up a general structural genomics laboratory information management system (LIMS). Our project recently turned towards the resolution of protein domains and we also started to work on membrane proteins. Strategies and first results will be presented.

O7-3

Biological Solid-State NMR Spectroscopy: Applications to Membrane Proteins

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The finding that tens of thousands of membrane proteins are encoded for by known genomes, many of which control vital biological functions, has increased the interest in establishing structure-function relationships for membrane proteins using solid-state NMR spectroscopy. With such motivations, solid-state NMR has undergone a tremendous evolution during the past few years, leading to improved instrumentation, the development and assembly of advanced solid-state NMR pulse sequences, and improved methods for data interpretation. Using state-of-the-art technology, it is now possible to handle responses from relatively large isotope-labelled peptides/proteins to establish information about the three-dimensional structure and molecular dynamics. At the same time, however, it is clear that even the best methods lack resolution and sensitivity compared to the corresponding liquid-state NMR technologies for similar size globular proteins, implying that structural analysis of, e.g., large membrane proteins still calls for considerable method development.
In this presentation we describe various elements in, and tools for, systematic method development in biological solid-state NMR along with practical demonstrations on membrane peptides/proteins. The latter will include NMR studies of the alamethicin ion channel, the M5 helix of Ca^{2+}-ATPase, and ^{15}N-labeled variants of the 7TM membrane protein bacteriorhodopsin.

O7-4

Folding intermediates in elongated variants of alfa-spectrin SH3 domain

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Amide hydrogen/deuterium (H/D) exchange rates have been determined for two mutants of alfa-spectrin SH3 domain (WT), containing an elongated stable (SHH) and unstable (SHA) distal loop. SHA, similarly to WT follows a two-state transition, while SHH apparently folds via a three-state mechanism. Native state amide hydrogen exchange (HX) results effective in ascribing energetic readjustments observed in kinetic experiments to species stabilized within the denatured base, and distinguishing those from high-energy barrier-crossings. Comparison of hydrogen exchange stabilities and their dependence with denaturant for amide protons of these mutants demonstrate the existence of an intermediate (I) and allows the identification of protons protected in this state. The consolidation of a form containing a pre-folded long b-hairpin induces the switch to a three state mechanism in an otherwise two-state folder. It can be inferred that the unbalanced high stability of individual elements of secondary structure in a polypeptide could ultimately complicate its folding mechanism.

Oral Presentations

- Protein structure, dynamics and functions (II)-

O7-5

Difference FTIR spectroscopy and hydrogen deuterium exchange to describe conformational changes in membrane proteins

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As more and more high-resolution structures of proteins become available, the new challenge is the understanding of these small conformational changes that are responsible for protein activity. Specialized difference FTIR techniques allow the recording of side chain modifications or minute secondary structure changes. Yet, large domain movements remain usually unsuspected. FTIR spectroscopy also provides a unique opportunity to record H/D exchange kinetics at the level of the amide proton. This approach is extremely sensitive to tertiary structure changes and yields quantitative data on domain/domain interactions [1]. An experimental setup designed for attenuated total reflection and a specific approach for the analysis of the results will be proposed. The study of two membrane proteins, the gastric H+,K+-ATPase [2] and the bacterial LmrA [3], will demonstrate the usefulness of difference spectroscopy methods and H/D exchange kinetics for the understanding of the conformational changes related to the catalytic activity. A new approach was developed in order to provide information about membrane domain dynamics. Monitoring the infrared linear dichroism spectra in the course of H/D exchange allowed focusing the recording of exchange rates on the membrane-embedded region of the protein only. This approach revealed an unusual structural dynamics, indicating high flexibility in this antibiotic binding and transport region of LmrA. [1] Grimard,V., C.Vigano, A.Margolles, R.Wattiez, H.W.van-Veen, W.N.Konings, J.M.Ruysschaert, and E.Goormaghtigh, Biochemistry 40 (2001), 11876-11886. [2] Scheirlinckx,F., R.Buchet, J.M.Ruysschaert, and E.Goormaghtigh, Eur.J.Biochem. 268 (2001), 3644-3653. [3] Goormaghtigh,E., V.Raussens, and J.M.Ruysschaert, Biochim.Biophys.Acta 1422 (1999), 105-185.

O7-6

Structural basis of tyrosinase function

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The structural basis that define the physiological functions of binuclear copper enzymes have been investigated using a broad spectroscopic approach that spans from paramagnetic NMR and pulsed EPR to x-ray absorption spectroscopies. As a paradigmatic example of this protein family the data obtained from bacterial (Streptomyces antibioticus) Tyrosinase will be presented. This enzyme, obtained from a genetically engineered system, has been investigated in the different oxidation state accessible to a binuclear copper site in order to unravel the structural details of its physiological functions. These include reversible oxygen binding and molecular oxygen activation for the conversion of monophenols and ortho-diphenols to the corresponding ortho-quinones. A molecular model will be presented to rationalize both the reaction mechanism of this class of enzyme and the basis for substrate specificity.

Oral Presentations

- Single molecule biophysics -

O8-1

Structural and conformational mechanics of single biomolecules

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Many proteins in our body have a mechanical function. Examples range from cytoskeletal and muscle proteins to the various classes of molecular motors that transport cargo within the cell. Only recently the development of ultrasensitive force probes with a high spatial resolution, like AFM and optical tweezers, has allowed us to measure the mechanical properties of single molecules directly. I will discuss mechanical unfolding experiments with titin, the myosin coiled-coil and the cytoskeletal actin crosslinker Ddfilamin. We find that the mechanical properties are dependent on the topology of the fold. Full elasticity and reversibility is found for simple folds like coiled-coils, whereas the unfolding of more complicated structures occurs in non-equilibrium.

O8-2

Single molecule force spectroscopy on DNA with magnetic tweezers

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O8-3

Structural and conformational mechanics of single biomolecules

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A detailed understanding of molecular processes is the basic requirement for the description of cellular function. These processes typically involve the interplay of different proteins, but also of proteins and the lipids in the cell membrane. While large scale rearrangements of the protein distribution proceed on seconds up to minutes time scales, local changes are much faster. New ultra-sensitive methodologies for imaging single molecules in living cells allow the direct observation of molecular rearrangements on millisecond time scales. Such rearrangements are of particular importance during the stimulation of immune cells. We followed the motion of individual molecules during different phases of T-cell stimulation in the area of the immunological synapse. The mobility of different proteins, as they pass the synapse, reveals information upon the microstructure of such cell-to-cell contact areas. Such studies shed further light on the structural relevance of lipid microdomains for T-cell stimulation. (supported by the Austrian Research Funds)

Oral Presentations

- Biomaterials and bionanotechnology -

O9-1

A new bioactive material: Bioeutectic®

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A new route of obtaining bioactive ceramic materials, which improve the ingrowth of new bone into implants (osseointegration), is presented. This involves obtaining eutectic structures from selected systems, bearing in mind the different bioactive behaviour of the phases. In this work the eutectic binary system wollastonite-tricalcium phosphate is resorbable. The eutectic material is formed by spherical colonies composed of alternating radial lamellae of wollastonita and tricalcium phosphate. The eutectic material, in in vitro experiments, reacts by dissolving the wollastonite phase and forming a porous structure of hydroxyapatite by a pseudomorphic transformation of the tricalcium phosphate lamellae, which in turn mimic porous bone. Later, a hydroxyapatite layer is formed by precipitation on the surface of the material. The procedure developed by the authors open the opportunity to obtain a new family of bioactive materials for which the general name of Bioeutectic® is proposed.

O9-2

DNA nanostructures: artificial crystals and synthetic motors

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DNA is a wonderful material for construction on a molecular scale. Its information storage capacity is the key to its usefulness: short strands of DNA can be designed to recognise and bind to each other to form complex three-dimensional structures that self-assemble with a precision unattainable by any form of lithography. I will describe periodic structures used as scaffolds for crystallography, and the use of DNA in the construction of functional molecular-scale devices including synthetic molecular motors.

O9-3

Biological applications of quantum dots

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Due to their interesting properties, research on colloidal nanocrystals (quantum dots) has moved in the last years from fundamental research to first applications in materials science and life sciences. We will discuss some recent biological applications of colloidal nanocrystals. First, the properties of colloidal nanocrystals and how they can be synthesized are described. Second, the conjugation of the nanocrystals with biological molecules is discussed. And third, three different biological applications are introduced: i) the arrangement of nanocrystal-oligonucleotide conjugates using molecular scaffolds such as single stranded DNA, ii) the use of nanocrystal-protein conjugates as fluorescent probes for cellular imaging, iii) a motility assay based on the uptake of nanocrystals by living cells.

O9-4

Osteointegration improvement using BMP2

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Bone reparation and regeneration is an important topic of actual interest in daily life. The use of several bone morphogenetic proteins (BMP) has focused the main clinical research interest due to its ectopic bone induction ability. These BMP play an essential role in dorsal-ventral patterning at vertebrates embryo level and mesoderm skeletal system development, they are expressed in several adult tissues and used by the organism to repair and regenerate bone and other tissues during postfetal life. For any possible application of these proteins to this aim is necessary to obtain them in enough amounts with the highest required activity and purity and to find a suitable carrier for its liberation at target location. Our research group expressed several years ago human recombinant BMP2 in a bacterial system (P 99900409). Nevertheless, for any practical application is necessary to find adequate carriers able to transport and liberate the protein in its active form at the desired anatomical localization. They must also be biocompatible, biodegradable and to have suitable mechanical properties, whilst simultaneously preserve the protein activity during the time required for osteointegration and bone reparation. In this communication we presents some results obtained in vitro and in vivo experiments performed with the rhBMP-2 produced in our laboratory and different biomaterial carriers tested, mainly chitosan (PCT/ES01/00322). The animal models tested so far are whistar rats (ectopic bone induction), New Zealand rabbits (bone regeneration and dental implant osteointegration) and dogs. Funding: CICYT Mat2001-1376, 2FD97-2034, Mat98-0702

Oral Presentations

- Biomaterials and bionanotechnology -

O9-5

Supramolecular asszebly of redox proteins and nucleic acids onto a supported bilayer.

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Development and characterization of highly organized nanostructures involving biological components onto an inorganic support is still a challenge. Most experiments involve direct immobilization methods leading to constrained geometries without possibility of self-reorganization after the initial assembling phase. We designed dynamic structures involving the co-organization of redox proteins and nucleic acids (P-DNA assembly) onto a supported lipidic bilayer. This original biodevice featured reversible and self-assembling properties allowing regeneration of the probe, thus contributing to its high potential as DNA sensorchip. A new P-DNA structure was designed with unique geometry and characterized by: a modified cytochrome b5 engineered by substitution of its C-terminal domain by a four-histidine-residue hydrophilic tail used for specific coupling to the lipidic bilayer and modified with a unique cysteine for the covalent coupling of a nucleotide probe an oligonucleotide modified with a terminal (or intramolecular) thiol (or amine) functional group and crosslinked to the redox protein via a bifonctional linker. Characterization of the structure by surface plasmon resonance showed that immobilization by the metal ion affinity (IMAC) principle can be used to build high affinity, specific and reversible interactions between P-DNA and a hybrid bilayer. Maximal SPR response reached three hundred RU and recovery was large enough to perform hybridization experiments of complementary oligomer. We demonstrated that the highly versatile systems obtained by this method, along with their ability to perform tuneable, reversible assembly in near physiological conditions, show great potential for a new generation of highly specific and sensitive DNA sensors.

Oral Presentations
- Ion channels -

O10-1

Known and putative physiological roles of ERG and ELK ion channels"

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The ether-a-go-go (*eag*) gene is the founding member of a family of K^+ ion channels in *Drosophila* and mammals with relatives that are *erg* (eag-related gene, *erg1, erg2, erg3*) and *elk* (eag-like, *elk1 elk2*). Among the tissues expressing ERG channels, the most studied example is heart, in which the rapidly activating K^+ current is responsible for the repolarization of the action potential; its prolongation produces severe syndromes (LQT), among which, one (LQT2) is due to mutations in *herg*. HERG channels may also sustain a process of spike-frequency adaptation, and thus contribute to controlling of burst duration in cerebellum Purkinje neurons, smooth muscle, carotid body, lactotrophs, human beta-pancreatic cells and rat chromaffin cells. Our laboratory have shown that ERG channels in a differentiated neuroblastoma cell sustain spike-frequency adaptation during long trains of spikes because, at rest, an outward ERG current develops that is sufficient to inhibit firing. ELK2 channels have been found in human astrocytoma cells but their role is unknown. It has been shown that also in the CNS the various members of these families are variably expressed but their roles are difficult to be described due to the lack of specific blockers.

O10-2

Intracellular chloride channels in neurodegeneration

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Intracellular chloride channels (iClC) belong to the voltage-dependent chloride channel family with ubiquitous tissue distribution. Although iClC structurally belongs to a family of plasma membrane proteins, they localize in different organelle. For instance, ClC-7 expresses in late endosomal and lysosomal membranes. Mice lacking ClC-7 have a severe osteopetrotic phenotype that seems to emerge from a deficiency in HCl-secretion by osteoclasts. ClC-7 knockout mice also display a neuronal phenotype (i.e. ataxia) and show intracellular deposits in neurons, which resemble those found in neuronal ceroid lipofuscinosis (NCL). ClC-3 is an endosomal membrane channel. ClC-3 deficient mice reveal conspicuous hippocampal degeneration but only marginal signs of NCL. In addition, ClC-3 and ClC-7 knockout mice display retinal degeneration beginning at ~P15 with shortening of the outer segments endosomal. Interestingly, the subcellular distribution of the ClC-3 channel appears shifted from endosomal to lysosomal compartments in *Clcn7*$^{-/-}$ mice.

O10-3

Functional and molecular characterization of a two-p-domain potassium channel in astroglial cells

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[1]Dept. of Human and General Physiology, University of Bologna, Bologna, Italy, [2]Institute of Biophysics, CNR, Genova, Italy, [3]Dept. of Neuromorphology, Max-Planck-Institute of Neurobiology, Martinsried, Germany.

Two-p-domain potassium (K^+) channels constitute a novel class of channel proteins expressed in several mammalian tissues. These channels are opened by a variety of stimuli, and in the central nervous system (CNS) they are thought to mediate the background K^+ conductance of different populations of neuronal cells. By contrast, little is known on their presence and functional role in non-excitable cells of the CNS. Here we show that in cultured astroglial cells the bioactive fatty acid arachidonic acid (AA) activates a K^+ channel, which resembles TREK/TRAAK members of this channel family. Activation of the channel occurred with latency, and was preceded by a partial depression of the delayed rectifier K^+ current. Changes in extracellular K^+ concentration revealed that the channel was an open rectifier as K^+ conduction approximated the constant-field current equation for free electrodiffusion. Pharmacological experiments showed that this AA-activated K^+ channel was weakly sensitive to classic K+ channel blockers, but was strongly inhibited by lanthanum and gadolinium ions. AA action was not mediated by the byproducts of AA metabolism, and implicated a direct interaction of AA with the extracellular side of the plasma membrane. Moreover, channel activity was also elicited by lysophospholipids including lysophosphatidylcholine. Finally, RT-PCR analysis indicated that cultured astrocytes express the transcript for the TREK-2 member of the two-p-domain K^+ channel family. The possible biological role and modulation of this novel K^+ conductance in astroglial cells will also be addressed.

O10-4

Three mechanisms underlie kcnq2/3 heteromeric potassium m-channel potentiation

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The non-inactivating potassium M-current exerts a strong control in neuronal excitability. It is made up by the combination of neuronal KCNQ subunits. Mutations on either KCNQ2 or KCNQ3 leads to Benign Neonatal Familial Convulsions. A mere reduction of 25% in KCNQ2/3 function can increase excitability to epileptogenic levels, whereas its potentiation have antiepileptogenic effects. Expression of KCNQ3 alone in Xenopus oocytes does not give rise to detectable currents, whereas co-expression with KCNQ2 leads to current levels ten fold larger than with KCNQ2 alone. We have conceptually divided the channel into three portions (N-terminal, Transmembrane and C-terminal) that were exchanged between KCNQ2 and KCNQ3. The chimaeras where co-expressed with KCNQ3, and the formation of heteromeric channels was monitored taking advantage of the difference in TEA sensitivity of KCNQ2 (IC50 ~ 0.2 mM), KCNQ3 (IC50 > 30 mM) and heteromers (IC50 ~ 3.5 mM). Surface expression of HA-tagged KCNQ3 was evaluated using a luminescent based assay. Our results reveal the existence of three processes:
1.- Surface expression is controlled by the C-terminal region, increasing when there is an heteromeric configuration. 2.- The N-terminal domain from KCNQ2 exerts a negative control, probably by reducing the maximum probability of channel opening. 3.- A residue in the inner vestibule located after the selectivity filter plays a critical role. Regardless of the N- and C-terminal configuration, expression of channels with an homomeric KCNQ3 pore configuration gave rise to negligible or no current, whereas heteromeric or KCNQ2 homomeric pore configurations allowed significant current flux. This work has supported by the UE and by a grant from the FIS.This work has supported by the UE and by a grant from the Fondo de Investigaciones Sanitarias.

Oral Presentations

- Ion channels (I)-

O10-5

Molecular mechanisms of general anesthetics: selective effects on Kv channels

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The mechanisms of general anesthesia are largely unknown. Although local anesthetics have general anesthetic effects, voltage gated channel block have been assumed not to play a role for general anesthesia. Recently, however, Shaw2 has been suggested to be a critical target for halothane and alcohols, and with a binding site on the S4-S5 linker. Previously, we have shown that the local anesthetic bupivacaine blocks Kv channels in open state. We have also shown that bupivacaine differentiates between Kv2.1 and other Kv channels (i.e. Kv1.1, 1.2, 1.5, 3.1 and 3.2) with respect to the channel closing, and that the S5-S6 linker affects binding. In the present study we have analysed the effects of the general anesthetics ketamine, propofol and butanol on Kv1.2, Kv2.1 and chimeras, expressed in Xenopus laevis oocytes. Ketamine and propofol selectively block Kv2.1, while butanol does not show selectivity. In similarity with local anesthetics, ketamine blocks the channels in open state, affects the Kv2.1 channel closing and depends on the S5-S6 linker for binding. In contrast, propofol blocks the channels in closed state and depends on the S4-S5 linker for binding. Blocking Kv channels may disrupt oscillatory activity patterns in cortical neurons, and different blocking mechanisms may cause drastically different modifications. The results suggest that the Kv effects may contribute to general anesthesia.

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Oral Presentations

- Biophysics and bioengineering of sensory systems -

O11-1

The temporal structure of signal processing in the retina

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The vertebrate retina forms an intricate network of neurons with feed forward, feedback, and lateral connections. Visual stimuli elicit temporally structured responses in the retinal interneurons. This structure depends on neuronal type and the stimulus configuration. Consequently, the output signal of the retina, formed by action potential trains of the ganglion cells, is also temporally structured. For the design of a bioinspired visual prothesis, this temporal structure should be considered. Intracellular and multi-electrode, extracellular recordings were performed from all major interneurons and large ganglion cell populations of the turtle retina. Various light stimuli were applied and latencies, time to response peaks and temporal structure of ganglion cell spike trains were analyzed. The complexity of the temporal structure in ganglion cell spike trains increases with intensity, spatial extent and duration of the stimulus. It´s fine structure probably results from spike refractory period, whereas the coarse structure correlates to certain response features of the various types of retinal interneurons. Some features of this structure can be modelled with a simple contrast-gain model which includes delayed feedback lines. Since this structure occurs within the first 300 ms of the responses, we suggest that it might play an important role for object recognition during fixation periods, which also range between 100 and 500 ms in humans. Supported by CORTIVIS and DFG Am70.

O11-2

Active detection of sounds in the inner ear

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Hair cells of the inner ear detect mechanical stimuli by deflections of the hair bundle, which open tension-gated transduction channels in the cell membrane to admit cations from the surrounding fluid. Recent experiments have shown that the hair bundle has an active response and is not just a passive elastic structure. Indeed, spontaneous oscillations of the bundle have been observed in the absence of a stimulus. We have proposed the general concept of 'self-tuned criticality' to explain why such oscillations occur, and how they help the ear to hear. According to this idea, when working normally each hair cell is maintained at the threshold of an oscillatory instability. Poised on the verge of vibrating at a characteristic frequency, a hair bundle is especially responsive to weak periodic stimuli at that frequency. The concept of critical oscillators also sheds light on the transmission of acoustic energy within the cochlea. Classic experiments have demonstrated that a sound stimulus entering the inner ear excites a deformation of the basilar membrane which travels towards the apex and reaches peak amplitude at a location that depends on the frequency. We have put forward a model in which critical oscillators are ranged along the membrane, and are positioned so as to drive its motion. The basilar membrane is then an excitable medium which propagates an active traveling wave. The resultant nonlinear response accords with many of the observed features of cochlear tuning.

O11-3

The photochemistry of photobiology, with PYP and AppA as prime examples

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To properly respond to changes in fluency conditions, Nature has developed a variety of photosensors that modulate gene expression, enzyme activity and/or motility. Dedicated types have evolved, which can be classified in six families: rhodopsins, phytochromes, xanthopsins, cryptochromes, phototropins and BLUF proteins. Surprisingly, the latter three all use flavin as their chromophore. The photochemistry of rhodopsins, phytochromes and xanthopsins is based on *cis/trans* isomerization of an ethylene bond. In the flavin-containing families, exciting new types of photochemistry have been discovered. The basis of signal generation within the xanthopsins will be illustrated via Photoactive Yellow Protein from *Ectothiorhodospira halophila*. Its activation proceeds through *trans/cis* isomerization of the 7,8-vinyl bond of its hydroxycinnamic acid chromophore. This initiates a large conformational transition, leading to a phototactic response of the bacterium. Photoactivation initiates a photocycle (Ö = 0.35) with several intermediates, like pR and pB, formed after H+-transfer from E46 to the chromophore. The negative charge of E46 in the interior of the protein causes destabilization and subsequent partial unfolding. Refolding kinetics is dependent on the mesoscopic context of the protein. Much less is known about the anti-transcriptional regulator AppA, a BLUF-family member from *Rhodobacter sphaeroides*. Nevertheless, initial characterization revealed that its photochemistry is based on lightinduced deprotonation of its FAD chromophore, forming a signaling state which recovers with a rate of ~ 10-3 s-1. Furthermore, also this photoreceptor is partially unfolded in its signaling state. It is a challenge to resolve the to resolve the role of these partially unfolded signaling states for biological signal transfer.

O11-4

The use of insects as biosensors for the detection of volatile compounds

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Insects have evolved exquisitely sensitive olfactory sensors on their antennae and can perceive, with great specificity, very low concentrations of airborne volatiles For example, electrophysiological recordings from single antennal cells can detect 108 molcules per ml air. We have used this to detect volatiles present at an early stage of food deterioration. Recordings from the antennae of the fruit fly, *Drosophila melanogaster* demonstrate that it has olfactory cells which can discriminate between a healthy fruit and one which, although visually perfect, will begin to deteriorate within a few days. The cell is so sensitive that it can detect the difference between two punnets of tomatoes containing either 24 firm fruits, or 23 firm fruits plus one in an early stage of deterioration (not visible to the human eye). Similar specificity and sensitivity were found for the blowfly *Calliphor vomitoria* with volatiles from fish or meat, either fresh or in an early state of deterioration. The insect systems offer the prospect of developing biosensors, which are widely sought by the food processing and retail industries for early warning of loss of quality and safety. A biosensor incorporating preparations from insect antennae could comprise either a live insect or, in the longer term, the proteins which confer the molecular recognition. To this end we have cloned and expressed genes encoding the binding proteins responsible for initial interactions with the volatile signal chemicals in the insect antennae. These proteins are small, water-soluble and highly stable molecules suitable for incorporation into biosensors with enormous potential for many industrial applications.

Oral Presentations

- Biophysics and bioengineering of sensory systems -

O11-5

Site-directed spin labeling reveals the structure and conformational changes of the sensory rhodopsins-transducer complex

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X- (9 GHz) and W- (95 GHz) band electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labeling and molecular dynamics simulations has emerged as a powerful method to study the structure and conformational dynamics of proteins [1-3]. The present study reports EPR based analyses of conformational changes of the halobacterial phototaxis receptor sensory rhodopsin II after light activation. This member of the archeabacterial rhodopsin familiy is receiving more and more interest because it can be considered as a general model system for transmembrane signal transduction. The signal is transferred across the plasma membrane by means of a receptor-specific transducer protein (HtrII) that binds tightly to the photoreceptor. Inter-spin distances determined from a set of 26 pairs of interacting nitroxide spin labels introduced into the pSRII-transducer complex lead to a unique structural model of the dimeric complex [4]. Time resolved detection of inter-spin distance changes after light activation reveals the mechanism of the signal transfer from pSRII to the associated transducer HtrII. 1. Rink, T., Pfeiffer, M., Oesterhelt, D., Gerwert, K., and Steinhoff, H.J. Biophys. J. 2000, 78, 1519. 2. Steinhoff, H.-J., Savitsky, A., Wegener, C., Pfeiffer, M., Plato, M., Moebius, K. 2000. Biochim. Biophys. Acta 1457, 253. 3. Radzwill, N., Gerwert, K., and Steinhoff, H.J. Biophys. J. 2001, 80, 2856-2866. 4. Wegener, A., Klare, J., Engelhard, M., Steinhoff, H.-J. 2001. EMBO J. 20, 5312-5319.

Oral Presentations

- Water transport across biological membranes: biophysics, regulation and molecular aspects -

O12-1

Structure, dynamics and mechanism of water permeation through aquaporins

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A model of the structure of human Aquaporin-1 was built based on the x-ray structure of the bacterial glycerol facilitator GlpF, and subsequently refined against cryo-electron crystallographic data at 3.8 Å resolution [1]. A detailed structure comparison [2] showed a remarkable similarity to the recently solved x-ray structure of bovine aquaporin-1. `Real time' molecular dynamics simulations of water permeation through the pores of both aquaporin-1 and the homologous bacterial glycerol facilitator GlpF were performed, from which a time-resolved, atomic-resolution model of the permeation mechanism across these highly selective membrane channels was obtained. Both proteins act as two-stage filters: conserved fingerprint (Asparagine-Proline-Alanine, NPA) motifs together with a second (`aromatic/Arginine') region jointly enable the selective, yet efficient permeation of water and linear alcohols, respectively [3].

References:
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[2] Bert L. de Groot, Andreas Engel, and Helmut Grubmüller; "The structure of the Aquaporin-1 water channel: a comparison between cryo-electron microscopy and x-ray crystallography." J. Mol. Biol. 325: 485-493 (2003)
[3] Bert L. de Groot and Helmut Grubmüller; "Water Permeation Across Biological Membranes: Mechanism and Dynamics of Aquaporin-1 and GlpF"; Science 294:2353-2357 (2001).

O12-2

The cell biology of the Aquaporin-2 water channel: to pee or not to pee

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In mammals, vasopressin (AVP) regulates the osmotic water balance through its action in renal collecting ducts. With hypernatremia or hypovolemia, AVP activates intracellular cAMP and Ca2+ signaling cascades, resulting in a translocation of the homotetrameric Aquaporin-2 (AQP2) water channel from intracellular vesicles to the apical membrane. Consequently urine is concentrated. For this translocation, phosphorylation of AQP2 at Ser256 is essential, but other mechanisms can overrule this phosphorylation-denpendent translocation. Mutations in the AQP2 gene cause autosomal recessive and dominant Nephrogenic Diabetes Insipidus (NDI), a disease in which the kidney is unable to concentrate urine upon stimulation with AVP. Interestingly, all mutations in recessive NDI are within the core region, while mutations in dominant NDI are in the C-terminus. Cell biological studies revealed that all AQP2 mutants in recessive NDI are misfolded, retained in the endoplasmic reticulum and not able to tetramerise with wild-type (wt) AQP2. Analysis of the trafficking of some of the AQP2 mutants in dominant NDI revealed that these mutants are properly folded, but are missorted to late endosomes and the basolateral membrane, due to in the introduction of (mis)sorting signals in the mutant AQP2 proteins. Since these mutants do form heterotetramers with wt-AQP2, wt-AQP2 is also missorted. This results in a lack of wt-AQP2 in the apical membrane, which is completely consistent with the dominant nature of inheritance of NDI. In this seminar, I will present new insight into the regulation of wt-AQP2 and on the molecular mechanisms why AQP2 mutants cause dominant NDI.

O12-3

Water transport by cotransport proteins

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Cotransporters of the symport type, e.g. K-Cl, H-lactate, Na-glucose (SGLT1) and Na-glutamate, support both active and passive water transport: during cotransport water is transported actively along with the substrates, say Na and glucose in SGLT1; exposed to osmotic gradients the cotransporters function as water channels. The physiological role is important: first, the unit water permeability of cotransporters is about one tenth of that of AQP1 and similar to that of AQP0. Second, the number of cotransporters in a given membrane is high, dictated by, say, requirement for nutrients. In the brush border of the small intestine and of the kidney proximal tubule one third of the water uptake could be active while the remainder is osmotic, shared between the cotransporters, the lipid, and the aquaporins. Unstirred layer effects do not violate these estimates: the intracellular diffusion coefficients of the substrates are large, about half of those in free solutions. We are presently testing the two isoforms of the Na-K-Cl cotransporter: NKCC1 is ubiquitous in secretory cells, while NKCC2 transports ions across the water impermeable thick ascending limb of Henles loop. Accordingly, we find (Hamann et al., this meeting) that NKCC1 is water permeable and supports active water transport, while NKCC2 is water impermeable. The two isoforms are closely related; the major difference is the large intracellular amino-terminal of the NKCC1 that is absent in NKCC2. A comparison between the two isoforms gives a unique opportunity to study the molecular mechanism behind the water transport properties.

O12-4

Function, structural properties and molecular model of a bacterail aquaglyceroporin

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MIP proteins (Major Intrinsic Proteins) constitute a widespread super-family of transmembrane channels, essential for osmotic cell equilibrium and for metabolism. The MIPS can be classified into three major functional subgroups including aquaporins (AQPs) or specific water channels, glycerol facilitators (GlpF) permeable to small molecules such as glycerol, and aquaglyceroporins a class of glycerol facilitator also significantly permeable to water. All MIP channels share a common topology consisting of six transmembrane helices connected by five loops. Two small hydrophobic helices play an essential role in the pore formation and selectivity. The 3D structure of the human and bovine aquaporin AQP1 and of the GlpF of E. coli has been recently published at 2.2 angstroms of resolution. Both molecules exhibit a similar homotetrameric structure in which the selectivity properties could be partly explained by the nature of the residues located in the centre of each monomer. Whereas the tetrameric organization seems to be the functional form of aquaporins in biological membranes, in contrast, the functional organization of GlpF is disputed. We have observed, as others, that E. coli GlpF exists as multiple oligomeric states in the membrane, suggesting that functional specificity of MIPs is correlated to strength of self-association of monomers in the membrane bilayer. In the present study, we investigated the oligomerization state of GlaLlac, an aquaglyceroporin from Lactococcus lactis. We have also studied the nature of the residues that could be involved in the functional/structural properties of this MIP.

Oral Presentations

- Water transport across biological membranes: biophysics, regulation and molecular aspects -

O12-5

Liquid-vapour oscillations of water in hydrophobic nanopores

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We investigated the effect of quasi one-dimensional confinement on the structural, kinetic and transport-related properties of water in short (0.8 nm) hydrophobic pores at pore radii ranging from 0.15 nm to 1.0 nm by molecular dynamics simulations. These pores serve as models for a wide range of more complex naturally occurring pores like biological water and ion channels, carbon nanotubes, and zeolites. The observed water density in the pore fluctuates on a nanosecond time scale. In long simulations (more than 500 ns in total) we quantify the kinetics of these oscillations between a liquid-filled and a vapour-filled cavity. This behaviour can be explained as capillary evaporation alternating with capillary condensation, driven by pressure fluctuations in the water outside the pore. The free energy difference between the two states depends linearly on the radius. The free energy landscape shows how a metastable liquid state gradually develops with increasing radius. For radii larger than a critical radius of ca. 0.55 nm it becomes the globally stable state and the vapour state vanishes. One dimensional confinement affects the dynamic behaviour of the water molecules and increases the self diffusion by a factor of two to three compared to bulk water. Permeabilities for the narrow pores are of the same order of magnitude as for biological water pores even though water flow is not continuous but occurs in bursts. The behaviour of the water-pore system depends critically on the strength of the wall-water interaction.

Oral Presentations

- Membrane structure and dynamics -

O13-1

Structure and simulated function of bacterial porins

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Porins allow the passage of solutes across the outer bacterial membrane. Crystal structures show that the scaffold of the pore is a hollow beta-barrel constricted by inwardly folded loops that are forming part of the pore lining. The pore constriction of non-specific OmpF porin is highly charged and shows segregation of basic and acidic residues that face each other across the pore. A multitude of mutations have been analyzed functionally as well as structurally to get insight into the role of this peculiar charge constellation. Brownian dynamics simulations of ion translocation show good agreement with experimental cation/anion selectivity and relative single channel conductance (1). The simulations reveal distinct trajectories for cations and anions due to the dipolar potential at the pore constriction. The pore seems to be optimised for high throughput and low selectivity. The pore of the maltodextrin specific maltoporin (LamB) reveals an extended hydrophobic patch formed by six aromatic residues at the channel lining. As shown by X-ray crystallography maltodextrins bind to this 'greasy slide' at the height of the channel constriction. The 'Conjugate Gradient Method' has been employed to simulate the "register shift" of a maltohexaose sugar (2). The results show that no large energy barriers have to be overcome, despite the presence of a multitude of protein - sugar hydrogen bonds.

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2. Dutzler, R., Schirmer, T., Karplus, M. & Fischer, S. (2002). *Structure* 10, 1273.

O13-2

Long range conformational change between the membrane and the cytosolic domains of Multidrug Resistance Proteins

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Purified Human Multidrug Resistance Protein 1 (MRP1) is reconstituted into lipid vesicles with an inside-out orientation. Using hydrogen/deuterium exchange kinetics and limited trypsin digestion, the structural changes associated with each step of the drug transport process are analyzed (1). Drug binding induces a restructuring of MRP1 membrane-embedded domains that does not affect the cytosolic domains. On the opposite, GSH binding induces a conformational change that affects the structural organisation of the cytosolic domains and enhances ATP binding and/or hydrolysis suggesting that GSH mediated conformational changes are required for the coupling between drug transport and ATP hydrolysis. It is suggested that ATP hydrolysis might change the affinity of MRP1 for its substrate through helix rotation in the drug-binding domain. This step allows the release of the drug into the external medium. This approach can be extended to other ABC transporters that have been reconstituted and exhibited both ATP-dependent drug transport and drug-stimulated ATPase activity

1)Manciu, L., Chang, X.B., Buyse, F., Hou, Y.X., Gustot, A., Riordan, J.R., Ruysschaert, J.M. *J. Biol. Chem 2003,278(5):3347-56.*

O13-3

Solid-state NMR Studies on bacterial multidrug efflux pumps

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Membranes form the interface for cellular communication and provide entry and exit sites for proteins and solutes. Recently sequenced bacterial genomes have revealed that 3-10% of open reading frames are predicted to encode membrane transport proteins. These transporters are vital for cell nutrition, environmental sensing, ATP synthesis, protein/toxin secretion as well as influx and efflux of solutes. Some of these efflux pumps exhibit unusual broad substrates specificity thereby providing a mechanism for antibiotic resistance. However, the molecular mechanism of substrate recognition, discrimination and translocation across the membrane is not yet understood. Multidrug transport proteins are found in prokaryotic as well as eukaryotic organisms and across all known transporter families.
Here, we present first data on the *E.coli* transporter SugE, a member of the SMR family. Amplified expression in vivo and in vitro can be achieved at amounts needed for NMR spectroscopy. Solid-state NMR is used to study purified protein reconstituted into lipid bilayers which is essential to retain the protein in its functional form. Both MAS-NMR as well as NMR on strongly aligned samples are employed. In addition, Laser-CIDNP provides additional information on the accessibility of aromatic amino acids on the membrane surface.

O13-4

"Staying alive - evidence on how cell's maintain their lipid membrane"

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If one examines the lipid composition of cells it is striking that many of the components, on their own, will not give rise to a stable, impermeable bilayer membrane. All the evidence points to the membrane lipid composition of a cell being controlled and regulated by some homeostatic feedback mechanism that is able to sense the physical state of the lipid bilayer. We have explored the proposition, first made by Gruner, that it is the stored curvature elastic energy that is sensed by the homeostatic control mechanism. We have been able to show that the activity of the extrinsic membrane protein CTP:phosphocholine cytidylyltransferase is modulated in a predictable fashion by the stored curvature elastic stress. We have now gone on to investigate the competing effect of membrane surface charge density and have observed that, within physiological limits, the energetic effects of curvature elasticity are of greater significance than the effects of charge. The picture that emerges of cellular control of membrane composition will be compared to lipid compositional data from extremophiles.

Oral Presentations

- Membrane structure and dynamics -

O13-5

Structure and function of SNARE-proteins studied on "unroofed cells"

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During neuronal exocytosis, the vesicle-bound SNARE synaptobrevin 2 forms complexes with the plasma membrane-bound SNAREs syntaxin 1A and SNAP-25 to intiate the fusion reaction. We have studied the spatial organisation and the reactivity of the SNAREs in the plasma membrane of PC12 cells. To this end, flat plasma membrane sheets were generated by ultrasound treatment of intact cells. On the membrane sheets, SNAREs and secretory granules were visualized by immunostainings or by GFP-tags and their distribution was analysed with the fluorescence microscope. We found that secretory granules dock and fuse at special sites in the plasma membrane. The fusion site is a discrete, several hundred nm large area in the plasma membrane at which several thousand molecules of syntaxin 1A are concentrated. These syntaxin-clusters also contain cholesterol, that is required for their integrity. Cholesterol depletion, and hence syntaxin-cluster dispersal, leads to a strong inhibition of exocytic activity. The syntaxin molecules within the clusters are highly reactive and bind, in conjunction with membrane resident SNAP-25, added fluorescent labelled synaptobrevin 2. On freshly prepared membrane sheets, the vast majority of the syntaxin molecules can be cleaved by Botulinus neurotoxin C and hence is not engaged in ternary SNARE complexes, however, SNARE complexes are formed when the membrane sheets age. Formation of SNARE complexes between endogenous SNAREs is slow as it can be readily competed for by low concentrations of recombinant SNAREs. This points towards a control of protein activity in the membrane by segregating them into different microdomains. We conclude that calcium triggered fusion of secretory granules requires the presence of higly concentrated, reactive syntaxin molecules and cholesterol at the plasma membrane. Spatial

Oral Presentations
- Molecular machines -

O14-1

Structure and function of a folding nanomachine: the cytosolic chaperonin CCT

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Chaperonins are part of the larger family of molecular chaperones, a group of proteins devoted to assisting in the folding of other proteins. Chaperonins are large oligomers composed of two rings, each one encircling a cavity where folding takes place. However, the folding mechanism can be very different. In most chaperonins including GroEL from *E. coli*, the mechanism is a passive one: the unfolded polypeptide is trapped by a hydrophobic domain and then liberated in the cavity, which is locked with the help of a small oligomer named cochaperonin (GroES in the case of *E. coli*). In the cavity, free of any undesired interaction, the polypeptide can attain by itself its native conformation. In the case of the cytosolic chaperonin CCT, the mechanism is a more active one and this chaperonin could be considered a folding machine. Electron microscopy and biochemical studies have shown that CCT traps folding intermediates of specific proteins (actin and tubulin among others) and forces their folding using the conformational changes undergone by the chaperonin upon nucleotide binding.

O14-2

Measurement of strain-dependent kinetics of single myosin moleclules

Veigel C.
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O14-3

Macroscopic manifestations of molecular motors

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O14-4

Kinesin studied with single-pair forster resonance energy transfer

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Kinesin is a two-headed motor protein that moves processively along microtubules. This movement is powered by the hydrolysis of ATP. The binding and release of ATP, Pi and ADP lead to conformational changes that drive motility. However, the details of the conformational dynamics, the mechanism of stepping and the way the two motor domains are synchronized are still unclear and therefore precise single-molecule experiments on the conformational dynamics are required. We set out to measure the relative distance between the two motor domains in different nucleotide-bound states and during motility using FRET. For this purpose we expressed a dimeric, single-cysteine kinesin construct and labeled this with a different fluorophore on each head. These experiments are performed on a wide-field, epi-illuminated fluorescence microscope that is able to simultaneously image donor and acceptor emission from a single FRET-pair. We show that we can measure FRET between the two motor domains of single kinesin motors bound to microtubules in different nucleotide states.

Oral Presentations
- Molecular machines -

O14-5

Energy transmission in F1-ATP synthase by molecular stimulations

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The mitochondrial membrane FoF1-ATP synthase synthesizes adenosintriphosphate (ATP), the universal currency of energy in the cell. This process involves mechano-chemical energy transfer from the rotating gamma-stalk to the three active sites in F1 [1]. The ATP binding affinity is reduced and the bound ATP driven out of the binding pocket. Using molecular dynamics simulations we studied the structural changes and dynamics induced by the enforced rotation of the gamma-subunit in the F1-head, which mimics the effect of proton motive c-ring rotation in Fo during ATP synthesis. We identified propagating conformational motions along two routes leading to a lowering of the ATP affinity in the beta-TP binding pocket. For these motions, different time scales are found, which allows the separation of nanosecond from microsecond conformational motions. The affinity decrease in the beta-TP binding pocket is caused by a sequential and well-concerted retraction of three arginine residues from the bound ATP. Additionally, a fast, spontaneous closure of the empty beta-E subunit was found. This, together with longtime simulations performed on the isolated beta-subunit in different conformations and with different nucleotide occupancies, lead us to propose a new model for the transmission of torque for both the ATPase- and the ATP synthase direction. This results in a new binding change mechanism which is able to explain the reduced hydrolysis rate of gamma-depleted F1-ATPase and the measured high mean nucleotide occupancy. [1] J. P. Abrahams, A. G. W. Leslie et al. (1994) Nature, 370, 621-628 [2] R. A. Bockmann and H. Grubmuller (2002), Nature Struct. Biol., 9, 198-202

O14-6

Instabilities in muscle transient response

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We study a stochastic model for muscle myosin based on the swinging lever arm hypothesis and a four-state mechanochemical cycle. At thelevel of a single actin-myosin filament pair we show that when values of parameters such as the lever arm displacement and the crossbridge elasticity are chosen to provide a realstic value for the efficiency of energy transduction, the system shows a dynamical instability. This leads to a region of negative slope in the T2 curve (the tension recovered immediately after a length step) and under certain conditions to the generation of oscillations. If filament compliance and discrete binding sites are taken into account, the negative slope is diminished,but not eliminated. However, when the symmetric nature of whole sarcomeres is taken into account, filament rearrangement becomes important: as tension is recovered, some half-sarcomeres lengthen while others shorten. This leads to a flat T2 curve, as observed experimentally. The oscilations are not observable in general, unless the filaments get synchronised for a short time by a sudden change in applied force. These findings are in agreement with recent experiments by Edman and Curtin. We also investigate the effect of an inertial load, which can also lead to synchronisation of sarcomeres and suggest that this is the case in insect flight muscles. We conclude that it is essential to consider the collective dynamics of many sarcomeres, rather than the dynamics of a single pair of filaments, when interpreting experiments on muscle fibres.

Oral Presentations
- Macromolecular assemblies -

O15-1

Cryo-EM and 3-D analysis of microtubules and associated proteins .

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O15-2

Nucleoplasmin phosphorylation: structural basis for protein activation

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Nucleoplasmin (NP) is a key element in chromatin assembly and sperm chromatin remodeling. It belongs to a family of acidic proteins, and was the first identified molecular chaperone due to its ability to mediate nucleosome assembly. NP is pentameric, each monomer consisting of two domains: a structured core that forms a stable ring-like pentamer, and a disordered tail. The biological function of the protein is modulated by phosphorylation at multiple sites. We have been trying to understand the effect of this post-translational modification on the conformation and stability of this protein, in an attempt to establish a structure-function relationship. To this end, we have designed and characterized several mutants, and compare their biological activity and conformation with those of the active, natural proteins purified from *Xenopus laevis.* The conclusions derived from this work are the following: 1) Phosphorylation does not significantly affect the secondary structure of the protein but markedly decreases its stability. 2) Deletion mutants of the protein indicate that electrostatic interactions at the tail domain control the exposure of a binding site(s) for basic proteins. 3) The isolated core domain of the hyperphosphorylated protein, e.g. that extracted from eggs of *X. laevis*, is able to bind basic proteins and decondense chromatin. This activity can be artificially reproduced by replacing putative phosphorylation sites for aspartic acid. We proposed that phosphorylation might activate the protein by the generation of a localized negative potential in the core domain that contributes together with the tail domain in displaying a binding surface for basic proteins on the NP pentamer.

O15-3

The endothelial adhesive receptor VE cadherin: self assembly and biological properties

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Vascular Endothelial (VE) cadherin is an endothelial-specific adhesive receptor which establishes, via its extracellular part, homophilic interactions resulting in cell-cell attachment. These ectodomain-based interactions are reinforced by interactions involving the cytoplasmic domain of VE cadherin which binds intracellular α, β and γ catenins thus promoting connections between the actin cytoskeleton and the VE cadherin complex.

Recently, we presented evidence that the extracellular part of VE cadherin associates as a Ca^{++}-dependent hexamer in solution. Our results also suggest that, before elaborating a hexameric structure, VE cadherin molecules self-assemble as intermediate dimers. We also elaborated mutants which have lost the capacity to self associate as hexamers but form dimers. Expressed in cells, the impact of these mutations is drastic with respect to the formation of adhesive contacts. Indeed, we demonstrated that the VE cadherin-based hexameric structure is absolutely required for the elaboration of stable contacts between endothelial cells.

We collaborate with the laboratory of Pr A. Brisson (Lab. Imagerie Moléculaire et Nano-Bio-Technologie, LIMNT, IECB, Bordeaux), to reconstitute adherens-like junctions *in vitro*. This was performed using liposomes grafted with the His-tagged extracellular part of VE cadherin by nickel-chelating lipids. Cryo-micrographs of these junctions showed that VE cadherin fragments associate in an anti-parallel way at the surface of adjacent liposomes and constitute large structures mimicking cell-cell junctions.

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O15-4

Electron microscopy and 3D reconstruction of DNA repair kinases ATM and DNA-PK

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Human ATM and DNA-PKcs proteins are family-related kinases implicated in the detection and repair of breaks in the double-stranded DNA. Mutations in both kinases are linked to several tumours and disorders. These proteins are made of high molecular weight polipeptides (370 and 465 kDa, respectively) that share a limited homology restricted to the C-terminal region, where the kinase domain locates. ATM is rapidly activated after DNA damage by auto-phosphorylation, and it then phosphorylates other targets implicated in cell cycle arrest and DNA repair. DNA-PKcs is essential for the repair of double-stranded DNA breaks through non-homologous end-joining (NHEJ), and it also participates in V(D)J recombination. We have performed 3D reconstructions of purified human ATM and DNA-PKcs from single particle electron microscopy images. Despite their lack of homology in most of their sequence, the volumes of both kinases display a similar set of domains. Structural differences are found in regions probably relevant for specific functions. We have begun the structural analysis of some macromolecular assemblies implicated in the DNA repair process mediated by these kinases. A 3D structure of ATM incubated with a short DNA segment shows that it undergoes deep conformational changes in an arm-like domain that seems to play an important role during the DNA recognition process.

Oral Presentations

- Macromolecular assemblies -

O15-5

Intra and inter-nucleosomal dynamics

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In the nucleus of eucaryotic cells, DNA is wrapped around octamers of histones to form nucleosomes. The linear array of nucleosomes thus obtained undergoes, under the action of other proteins, various stages of compaction to form the chromatin fiber. It is now clear that chromatin structure and dynamics play a critical role in gene regulation. We're currently studying, by both experimental and theoretical approaches, the mechanisms underlying this dynamics at different levels. First, using an original technique of reconstitution of mononucleosomes on short linear or circular DNA fragments, we've shown that, far from being a "repetitive unit" of chromatin, each nucleosome has its own characteristics which are in particular conferred by the sequence of the wrapped DNA (ref. 1 and 2). Furthermore, by computing the elastic properties of chromatin fiber, we found that the way nucleosomes can change conformation and/or interact with neighboring nucleosomes can greatly influence the upper structure of chromatin (ref. 3 and 4). Hence, from "free" linear DNA to compact protein-DNA complex that form chromosomes, numerous steps occur that each depend on various constraints (mainly elastic and topological constraints) and interactions (with many different proteins). Both in vitro nucleosomes reconstitutions as well as molecular modeling and simulation should continue helping to get more insights about the mechanisms governing these steps. Ref 1. Sivolob, A. Lavelle, C. and Prunell, A. (2003). "Nucleosome dynamics. Sequence-dependent polymorphism. Potential involvement of histone H2B N-terminal tail proximal domain." J. Mol. Biol. 326:49-63. 2. Lavelle, C. and Prunell, A. (in preparation). "Nucleosome dynamics. Positioning-related nucleosome sequence-dependent polymorphism and stability."

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O15-6

The role of lipid-DNA interactions in nuclear envelope assembly

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Our earlier studies showed that the formation of the triple DNA-phosphatidylcholine liposomes-Me2+ complex was accompanied by aggregation and a partial and reversible fusion of liposomes, DNA acting as fusogen partially unwind during complex formation. The addition of an extract from the eggs of Xenopus laevis or Drosophila melanogaster to the triple complex resulted in a full fusion of small (100-200 nm) liposomes into giant liposomes 100 to 500 mkm in diameter. The addition of phosphatidylcholine liposomes to the extract from Xenopus laevis eggs gave rise to nuclear pore-like (annulate lamellae) or prepore structures in the lipid bilayer of liposomes. The pore diameter was approximately 2 times less than that of the native nuclear envelope, presumably due to the lower surface tension of lipid bilayers. This fact is in agreement with the earlier proposed model for the formation of nuclear pores as a result of lipid-DNA interactions (V.V. Kuvichkin Bioelectrochemistry 58 (2002) 3– 12).

Oral Presentations
- Cellular biophysics -

O16-1

Dynamic studies of membrane structure and endocytic processes using fluorescence correlation spectroscopy

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Dynamic membrane microdomains ("rafts"), which are operationally defined by their biochemical detergent-resistance, may play a crucial role in cellular processes involving membrane constituents, like signal transduction and vesicular transport. Fluorescence Correlation Spectroscopy is presented as a new approach that complements biochemical and microscopic techniques for studying endocytosis and membrane organization. We have shown that Dual-Color Fluorescence Cross-Correlation Spectroscopy can be used to follow endocytosis of cholera toxin mocules in live cells, where the receptor-binding B subunit and the catalytical A domain have been labelled with distinguishable fluorescent dyes. The subunits are observed to separate only after reaching the Golgi apparatus. Using a mixture of toxin molecules labelled with two different single colors, we show that the colocalization of differently labelled cargoes in the same small endocytic vesicles is detected with high sensitivity by cross-correlation spectroscopy. Applying this principle to two endocytic cargoes that bind to distinct receptors, we show their endocytic colocalization. Differences in mobility of a lipid phase marker and receptor-bound cholera toxin as measured by Fluorescence Autocorrelation Spectroscopy in artificial membranes and cell membranes support the notion of "lipid rafts" that has largely been built on biochemical detergent-insolubility.

O16-2

Structural basis of microtubule dynamic instability

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Key to microtubule function is the regulation of polymerization-depolymerization by nucleotide hydrolysis in tubulin and by interaction with cellular stabilizers and depolymerizers. Our atomic model of tubulin has allowed us to establish the structural basis of nucleotide exchange and polymerization-coupled hydrolysis. Our recently refined and greatly improved model of the tubulin dimer has also given us very precise information on the interaction taxol with tubulin. By docking the atomic structure of the protofilament into a cryo-em reconstruction of the microtubule we produced a high resolution model of the microtubule that allowed us to propose a mechanism for hydrolysis-induced depolymerization and for the mode of action of the anticancer drug taxol. More recently single particle analysis of high-resolution images of microtubules has lead to a microtubule map at 8 Å resolution where alpha helices can be directly visualized. We are now studying the conformational changes in tubulin that occur during microtubule breakdown using "crystalline" forms of GDP-tubulin corresponding to closely packed helices that diffract to close to 15 Å. In the cell the dynamics of microtubules are regulated by the interaction of these polymers with different factors. We would like to understand how the Kin I family of kinesins, binds to tubulin and causes the disruption of the microtubule lattice. We have determined that this enzyme binds to a single protofilament, and that its depolymerizing action requires the C-terminal tail of tubulin. We are now using cryo-electron microscopy and image reconstruction to characterize the structure of XKCM1-tubulin complexes.

O16-3

Redistribution of Ca^{2+} among cytosol and organella in excitable cells

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Recent results indicate that Ca^{2+} transport by organella contributes to shaping Ca^{2+} signals and exocytosis in excitable cells. Therefore, accurate measurements of $[Ca^{2+}]$ inside organella are essential for a comprehensive analysis of the Ca^{2+} redistribution upon stimulation. We have monitored stimuli-induced, subcellular Ca^{2+} changes inside organella by means of bioluminescence imaging of targeted aequorins. We find that Ca^{2+} entry through voltage-gated Ca^{2+} channels generates subplasmalemmal high-$[Ca^{2+}]_c$ domains, adequate for triggering exocytosis. A smaller increase of $[Ca^{2+}]_c$ is produced in the cell core, adequate for recruitment of the reserve pool of secretory vesicles to the plasma membrane. Most of the Ca^{2+} load is taken up by a mitochondrial pool close to the plasma membrane; the increase of $[Ca^{2+}]_M$ stimulates respiration in these mitochondria, thus providing local support for the exocytotic process. Relaxation of the $[Ca^{2+}]_c$ transient is due to Ca^{2+} extrusion through the plasma membrane. At this stage mitochondria release Ca^{2+} to the cytosol through the Na^+/Ca^{2+} exchanger, thus maintaining $[Ca^{2+}]_c$ discretely increased, specially at core regions of the cell, for periods of time that overlast the duration of the stimulus. Spontaneous or stimulated cell electric activity generated Ca^{2+} oscillations in cytosol, mitochondria and nucleus. Abolition of electric activity decreased mitochondrial NAD(P)H levels suggesting that the resting mitochondrial Ca^{2+} oscillations stimulate respiration. Whereas cytosolic Ca^{2+} oscillations were enlarged by mitochondria, they were dampened on propagation to nucleus. Thus, a single pattern of electric activity generated different patterns of Ca^{2+} oscillation in the cytosol, mitochondria and nucleus.

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O16-4

Probing single-ion channel dynamics

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By combining real-time single-molecule fluorescence imaging measurements with real-time single-channel current measurements in membranes of lipid bilayers or in living cells, it is possible to probe single ion-channel-protein conformational changes simultaneously correlated with single ion-channel current trajectories, providing an understanding at the molecular-level of the dynamics and mechanisms of ion-channel proteins. Subtle structural dynamics of ion channels play an important role in regulating channel function and selectivity. This technical innovation has been used to gain an understanding of how ion-channel activities are regulated by conformational change dynamics and assembly mechanisms. With the combination of single-molecule fluorescence and other existing single-molecule approaches, new tools can be developed to unravel the enigma of complex biological systems.

Oral Presentations

- Cellular biophysics -

O16-5

Secretory vesicle movements in living cells observed by TIRFM: Effect of Rab 27A and its effector MyRIPThe monomeric GTPase Rab27A has recently been seen

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The monomeric GTPase Rab27A has recently been shown to be present on secretory vesicles in chromaffin and PC-12 cells. In retinal pigment epithelial cells, Rab27A interacts with MyRIP (Myosin VII A and Rab Interacting Protein). This result suggests a functional association between vesicles and the actin cytoskeleton, through these proteins. To test this hypothesis, we analyzed the movements of secretory vesicles in the vicinity of the cell membrane using Total Internal Reflection Microscopy (TIRFM), also called Evanescent Wave Fluorescence Microscopy (EW-FM). PC-12 cells were cultured on a glass coverslip and their secretory vesicles labelled by transfection by NPY-GFP. The evanescent wave generated at the H2O/glass interface excited the fluorescence of vesicles close to the cell membrane (100-300 nm), in the subcortical actin network. Cells were co-transfected by NYP-GFP and GTPase-deficient Rab27A (Rab27A-Q78L) or various MyRIP constructs. The 2D diffusion coefficient (Dx,y) of the vesicles was derived from their trajectories obtained by single particle tracking. Distribution histograms of Dx,y for the various transfected cells were obtained. Cell expressing Rab27A-Q78L or overexpressing MyRIP had a decreased mobility of their vesicles, whereas those expressing the Rab27A binding domain of MyRIP displayed an increased mobility. We propose that MyRIP/Rab27 links the secretory vesicles to the actin cytoskeleton, probably via a non identified myosin.

Oral Presentations

- Transmembrane signalling -

O17-1

Membrane microdomains in signal transduction of immune cells .

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The plasma membrane is compartmentalized into microdomains and the association/dissociation of receptors and signaling molecules with/from these membrane domains appears to be a major principle for regulation of signal transduction. Thus, a detailed understanding of the molecular processes in microdomains is of basic requirement for the description of cell activation and function. Here we introduce single dye tracing for imaging molecular rearrangements in microdomains of living human blood T lymphocytes, the central cellular components of the specific immune response. Using this technique we observed on the single molecule level how microdomains reorganized upon T lymphocyte stimulation. Furthermore, we obtained evidence that not only positive regulating signals but also negative regulating ones modulate composition and reorganization of microdomains. Our data also indicate the existence of different types of microdomains. The microscopic data were confirmed by biochemical experiments. In summary we show in real time how microdomains reorganize upon T lymphocyte stimulation and provide evidence that membrane microdomains are also involved in signaling of negative regulating receptors in T lymphocytes.

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O17-2

Transmembrane Signaling in the Light of Electro-Immunology

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Biochemical and molecular biological events are fairly well explored in the best studied signal-transducing events in lymphoid cells, which are responsible for cellular- and humoral-immunity. Despite the well known facts that neither cellular nor humoral immune responses can be evoked in the absence of normal potassium channel activity in the lymphocyte membranes, little is known about further details of their actions on specific immune steps. Herewith we present data on the influence of genetically determined cell surface receptor distribution patterns, which are indicating some elements of the past and future of cells. Furthermore, data are presented, which clearly demonstrate non-random distribution of Kv1.3 type voltage gated potassium channels in plasma membrane of lymphoid cells. On the one hand, the ion channels have been found hetero-associated with CD3 receptors, which are important functional elements of the T-cell receptor. On the other hand, the T-cell receptors are the major instruments of cellular immunity in the formation of the immunological synapse. A variety of methods supported our view that ion-channel activities can influence immune reactions of competent cells even at the level of the immune synapse. The authors are aware of the implicit possibilities for pharmacological regulation of these important physiological and pathological events at cellular levels.

O17-3

Study of homoassociation of transmembrane receptors and RAS proteins using energy migration FRET (emFRET)

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Membrane proteins including transmembrane receptors and signal transducing molecules usually form homo- and heteroassociations. According to a long-standing dogma in cell biology peptide growth factors like epidermal growth factor (EGF) bind to receptor tyrosine kinases (RTK) and induce a dimerization-mediated activation. An RTK fused to a visible fluorescent protein (VFP; GFP and its spectral variants) can be used to study receptor associations in living cells. Although FRET between spectral VFP variants is feasible, it necessitates the transfection of two different vectors into a single cell even in the study of homoassociation. To circumvent this difficulty, we developed a microscopic and a flow cytometric approach in which "energy migration" FRET (emFRET) between like fluorophores is revealed by steady-state fluorescence anisotropy measurements. Whereas microscopy is able to collect data from dozens of cells with subcellular resolution, flow cytometry measures thousands of cells, although it lacks the resolution power of microscopy. We analyzed the fluorescence anisotropy of VFP fusion constructs and its dependence on the molecular number (density) expressed in single cells. EGFP, EYFP, and other VFPs were fused to RTKs (EGF receptor, erbB1; erbB2) and to ras, a low molecular weight G protein, and their homoassociation was analyzed using emFRET. We found evidence for ligand independent homoassociation of growth factor receptors and for a lipid environment dependent homoassociation of different ras isoforms. The implementation of emFRET by steady state anisotropy is relatively simple and holds a great potential for the study of receptor and other molecular homoassociations.

O17-4

Chemokine signaling and fuctional responses: Role of receptor dimerization and TK pathway activation

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The chemokines comprise a large family of low molecular weight (8-10 kDa) cytokines, with chemotactic and pro-activatory effects on different leukocyte lineages. Studies have established the central role of chemokines in a number of physiological situations, including T helper responses, hematopoiesis, angiogenesis, and homeostasis, as well as in pathological conditions such as asthma, tumor rejection, HIV-1 infection, and arteriosclerosis.
Chemokines signal through seven-transmembrane G protein-coupled receptors (GPCR) that transduce signals to a G_i protein. Association of $G\square_i$ with several chemokine receptors (CXCR1, CXCR4, CCR2 and CCR5) has been described; the majority of responses to chemokines are therefore inhibited by pertussis toxin (PTx). We observed that chemokine receptors are tyrosine phosphorylated following activation and that several Janus kinase (JAK) family members associate to the activated receptor independently of G_i.
Our laboratory has concentrated its efforts on understanding the mechanisms behind the activation of the tyrosine kinase pathway by chemokines, as well as its biological significance. Here, we focus on three aspects related with this JAK activation. First the study of ligand-mediated chemokine receptor dimerization, a phenomenon that has been observed in many receptor families, including the seven-transmembrane GPCR. Using several strategies we demonstrate that chemokine receptors dimerization is a critical step on chemokine signaling. Interestingly, in the presence of certain chemokine pairs, we have observed that it is also possible chemokine receptor heterodimerization

Oral Presentations

- Transmembrane signalling -

O17-5

Single molecule dynamics for signal transduction in the cell membrane

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We used single molecule techniques, including high resolution single particle tracking, single fluorescence molecule observation, and optical trapping, to detect rafts at resting state and to follow them after crosslinking or ligating the GPI-anchored protein CD59, triggering signaling cascades. Without stimuli, rafts appear to consist of only a few molecules; their lifetimes may be < 1 ms. Upon stimulation, rafts increase in size and undergo repeated transient immobilization by actin filaments induced by Src-family kinases (SFKs). Such temporary immobilization (average period = 0.73 s) turned out to represent formation of signaling rafts that are responsible for transducing the CD59 signal into intracellular calcium signaling. The second topic is visualization of temporal (< 0.6 s) activation of individual H-Ras molecules, which may be induced by cooperative assembly and disassembly of H-Ras, scaffolding proteins for activated H-Ras such as SUR-8, its downstream effector molecules like Raf, and possibly RasGAPs that deactivate activated H-Ras. In both cases, activation of single signaling molecules took place like a short pulse although signaling molecules collectively exhibit activation lasting over several minutes, a time course which is the same as that detected biochemically. It is concluded that these two cellular signaling systems adopt digital frequency-modulated signaling mechanisms.

Oral Presentations
- Ion channels (II)-

O18-1

Whole-cell potassium channel conductances in pancreatic tissue slice preparation

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O18-2

Structure-function correlation of a membrane protein in a ‚jig saw' approach: Vpu from HIV-1

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Structural information of a protein in general is a key step forward in elucidating the mechanism of function. Depending on the method applied, investigations on the whole protein can be demanding. We are therefore using only parts of the viral membrane protein Vpu from HIV-1 to gain an insight into the mechanisms of function of the full protein (a 'jig saw' approach). Vpu is a membrane protein of 81 amino acids encoded by HIV-1. Vpu function is to amplify the release of progeny virions via two distinct pathways: (i) docking of Vpu to CD4 with its cytoplasmic part in the ER and (ii) ion channel activity via self-assembly near the plasmamembrane. The structural motif of Vpu is: a helical transmembrane (TM) domain followed by a second helix (helix-2) parallel to the membrane surface, a third helix most likely in lose contact with the membrane and a short motif at the C terminal end either a turn or helix. Conductance measurements verify our computational data on the Vpu TM domain. This forms the basis for further investigation using MD simulations to predict structural stability of mutants and their potential for conducting ions (manuscript in preparation). In a next step we extend our simulations on a Vpu construct which includes the TM helix and a second helix (helix-2). Our simulations are indicative that helix-2 may act as a 'peptide float' riding on the lipid bilayer. Further simulations with helix-2 only support this idea (manuscript in preparation).

O18-3

Ca^{2+} regulation of exocytosis. Lessons from single vesicles and few ion channels

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Ca^{2+} microdomains formed during the opening of voltage-gated Ca^{2+} channels have been implicated in regulating the kinetics of hormone and transmitter release. Direct assessment of the interaction between a single Ca^{2+} microdomain and a single secretory vesicle has remained impossible due to technical limitations. Using evanescent-wave imaging of near-membrane micromolar [Ca^{2+}] and fluorescently labeled vesicles, we experimentally demonstrate Ca^{2+}-microdomain triggered-exocytosis of individual chromaffin dense core vesicles. Ca^{2+} microdomains selectively triggered release of a sub-population of vesicles docked within <300-nm distance. In addition to its established role as a trigger for release, elevated near-membrane [Ca^{2+}] reduced the distance between docked vesicles and Ca^{2+}-entry sites. Our results imply a new pathway of stimulation-dependent facilitation of exocytosis by moving vesicles close to a Ca^{2+}-entry site, thereby increasing a Ca^{2+} microdomain's efficacy to trigger vesicle fusion.

O18-4

Water transport across the KcsA channel

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A potassium ion requires 10 ns to pass through the KcsA channel, which is among the fastest ion channels (1). It is transported along with water by a single file mechanism, i.e. in the narrow part of the channel ions and water molecules cannot pass each other. To determine the rate at which water passes the channel, a large number of KcsA molecules was reconstituted into planar lipid bilayers. By means of scanning ion sensitive microelectrodes, the osmotic water permeability of the bilayer was obtained from the dilution of Mg or Ca ions within the hypotonic compartment immediately adjacent to the membrane (2, 3). The simultaneously monitored electrical membrane conductivity allowed to calculate the number of reconstituted KcsA channels. The resulting single KcsA channel water permeability exceeded the potassium permeability, suggesting that the potassium ions may be expelled from the channel. 1. LeMasurier, M., Heginbotham, L. & Miller, C. (2001) J. Gen. Physiol. 118, 303-314. 2. Pohl, P., Saparov, S. M., Borgnia, M. J. & Agre, P. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9624-9629. 3. Saparov, S. M., Kozono, D., Rothe, U., Agre, P. & Pohl, P. (2001) J. Biol. Chem. 276, 31515-31520.

Oral Presentations
- Ion channels (II)-

O18-5

Antibiotic translocation through ompf channel: a molecular dynamics investigation

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Protein channels serve to regulate the passage of small molecules and ions across membranes. Ompf from outer membrane of E. Coli is a general bacterial porin which is considered to be the main pathway for beta-lactam antibiotics. Recently new experimental techniques allowed to resolve individual molecule translocation through Ompf. Since Ompf is not specific for antibiotic transport, the investigation of the diffusion mechanism is an important step to improve the translocation of antibiotics at low concentration. Here we present a theoretical investigation, by means of molecular modelling at the atomic resolution, of the diffusion of an ampicillin molecule through the Ompf porin. We performed 10 ns of history-dependent molecular dynamics simulations(Laio and Parrinello PNAS 2002, 99: 12562) to force the diffusion process followed by 15 ns of equilibrium molecular dynamics to better characterize the most stable conformations sampled along the translocation. The atomic details of our model and the long simulations allowed us to find the affinity sites and the main interactions responsible of the diffusion process. We also compared our results with experimental measurements of the translocation of several antibiotics of the same family through wild type and mutated Ompf channels.

O18-6

A sequence in the proximal domain of Herg K^+ channel amino terminus is necessary for regulation by the phospholipase c-coupled Trh receptor

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The proximal domain located between the conserved initial amino-terminus and the first transmembrane helix is an important determinant of human ether-a-go-go related gene (HERG) channel activation gating properties (Viloria et al. Biophys. J. 79:231-246, 2000; Gomez-Varela et al. J. Membrane Biol. 187:117-133, 2002). The relevance of this domain for hormonal regulation of the channel by G-protein coupled receptors (Barros et al. J. Physiol. 511:333-346, 1998) has been explored using oocytes co-expressing TRH receptors and HERG channels carrying different amino-terminal modifications. Deletion of the whole proximal domain (delta138-373) eliminated TRH-induced modifications in activation and deactivation parameters. TRH effects on activation are also suppressed with channels lacking the second half of the proximal domain or only residues 326-373. However, normal responses to TRH are obtained with delta333-373 channels.This indicates that a seven amino acid sequence comprising residues 326 to 332 is required for the hormonal modulation of HERG activation. On the other hand, the TRH-induced effects on deactivation are totally recovered in channels lacking residues 284-373. Overall, our results indicate that different segments of the proximal domain contribute to set HERG activation gating and its hormonal regulation by TRH receptors, but also that different parts of this domain are involved in hormone-induced modification of activation and deactivation properties. Furthermore, they emphasize the important role of cytoplasmic N-terminal structures as regulators of channel gating and modulation by regulatory factors.