Posters
- Biomaterials and bionanotechnology -

9-1

A novel 2D invasion assay using quantum dot layers

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The ability of tumor cells to invade surrounding tissues and to metastasize to different sites is related with the cell motility. To test invasive capacities of different cancer cell lines we have developed a two dimensional invasion assay using water-soluble CdSe/ZnS nanocrystals. Semiconductor nanycrystals are inorganic fluorophores: excitation with UV light stimulates fluorescence in the visible range, whereby the color of fluorescence can be controlled by the size of the particles. It has been shown that cells are able to engulf nanocrystals in non-specific way. When cancer cells are seeded on top of a homogenous nanocrystals layer and are incubate at 37 oC for 24 h, they create trails free of nanocrystals that are no longer fluorescent. The size and the shape of these trails are related with the potential of invasiveness of the cells. We have compared the behavior of six different human cancer cell lines including colon, breast, lung and bone cancer cells. With this test it is possible to discriminate between non-invasive and invasive cancer cell lines.

9-2

Conjugation of biomolecules to silanized colloidal semiconductor nanocrystaline quantum dots

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Water soluble, highly fluorescent semiconductor nanocrystals are synthesized, coated with a glass shell, and functionalized with biological molecules. The functionalization is achieved by incorporating thiol, amino, or carboxyl groups to the outermost shell of the crystals. In a next step biological macromolecules are coupled to this shell via commercially available crosslinkers. When fed to cells one can observe the regions to which the biomolecules are transported by the bright fluorescence of the attached nanocrystals. This offers great possibilities for observing dynamic transport processes in living cells. Due to the small bandwidth of the nanocrystals' fluorescence line the location of several biomolecules inside the cell can be observed in parallel.

9-3

Protein-based integrated optical light-switch

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Devices used in telecommunications and data processing are of a hybrid nature, i.e. they consist of optical and electronic components, therefore their speed is limited by the relatively slow electronic components compared to the optical ones. Further development is going towards the production of faster components and there is a high demand for all-optical devices. Progress of development in the field of integrated optics allows us to make integrated optical circuits in comparable size to the conventional electronic ones. Since the production of these optical microstructures is well developed, our task is to find materials of proper nonlinear optical properties which could be used as active elements in these integrated optical devices. Besides organic and inorganic crystals, biological molecules are also considered to be used in molecular electronics and integrated optics, among them the chromoprotein bacteriorhodopsin generated the most interest. There are plenty of patents in the field of holography and sensory devices that utilize the favourable properties of this protein. Our aim was to develop an integrated optical, light-driven light-switch utilizing the nonlinear optical properties of bacteriorhodopsin. The light-induced refractive index change of the protein was measured by the Optical Waveguide Lightmode Spectroscopy technique, and was found to be comparable to, or even better than materials used in such applications. This result is very promising since it permits of producing a protein-based optically controlled light-switch which could be a basic element in integrated optical devices.

9-4

Electrodeless trapping of biomolecules by dielectrophoresis in a nanopipette

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We have previously reported that DNA in an aqueous environment can be delivered in a pulsed fashion using a nanopipette (about 50 nm radius) (Ying et al., Anal. Chem. 2002, 74, 1380-1385). The pulsed delivery can be achieved by using low applied voltages since the voltage drop and hence applied electric field occur only in the pipette tip. Here we have used dual colour fluorescence spectroscopy so that two different lengths of labelled DNA can be simultaneously observed in real time and with millisecond time resolution in the same nanopipette, allowing any difference in their behaviour to be observed. We have performed a detailed study of the voltage and frequency dependence of the trapping on 1kb, 40-base single stranded and double stranded DNA, and a single nucleotide (dCTP). We observed that DNA can be easily trapped at a negative potential during a half cycle and this was most effective at low frequencies and high applied voltages. No significant difference between the behaviour of double and single stranded DNA was observed suggesting that both molecules are aligned by the electric field in the pipette tip. We believe that the trapping is due to dielectrophoresis which occurs due to the high electric field and field gradient in the pipette tip. For the first time we have observed trapping of a short DNA (<50 bases) and even a single nucleotide inside such a nanostructure. This technique may find potential applications in concentrating and trapping DNA for analysis and in lab-on-the-chip applications.

Posters
- Biomaterials and bionanotechnology -

9-5

Exploring the nanomechanics of single virus shells using scanning force microscopy

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Viruses are the smallest forms of life and their construction is regular and well defined. The shell (prohead) of the bacteriophage phi29 virus we study is 50nm in diameter and can contain 20kbp DNA. To retain this amount of DNA at a pressure of 6Mpa, the shell must have a tensile strength of 100Mpa. To construct such a strong nanometer sized container, nature had to solve extraordinary design challenges. Using Scanning Force Microscopy (SFM) we have investigated the nanomechanical properties of the bacteriophage phi29 proheads. Since understanding this virus shell will not only teach us virus biology and the physics behind elastic (charged) shells, but will also give insight into Nature's engineering tricks on the nanometer scale and may have relevance for material design particularly in nanotechnology. In our SFM study of proheads we started with imaging them at high resolution in order to confirm the correct orientation and dimensions. Thereafter, by using an SFM as a force probe we have measured the elastic properties of these shell by direct deformation. These experiments indicate that a protein skeleton reinforces the prohead possibly due to the protein arrangement of the shell. This skeleton results in a shell which is resistant to collapse and is able to withstand pressures of 20Mpa. Nevertheless repeated pushing on a prohead will eventually cause breaks in the structure and over time this results in the complete destruction of the object. Using finite element analysis a lower limit of the Young's modules of the prohead is 1.3Gpa.

9-6

Thiocytosine as a hole trap in a system of stacked bases

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It has been shown that thiocytosine is a good hole trap when replacing cytosine in the DNA model systems. The molecular systems of selected nucleic-acid constituents offer a variety of base arrangements, and make good model systems for studying the mechanism and the migration range of electrons and/or holes. The study at 15 K revealed that in single crystals of cytosine monohydrate the holes induced by ionizing irradiation migrate to the distance of 1.0 - 1.5 nm in all directions before they are trapped. In thiocytosine-doped cytosine crystals unproportionally large amount of trapped hole radicals is associated with thiocytosine, as N1-deprotonated neutral species. After precise ENDOR study of the inter- and intra-molecular spin interactions, the Density Functional Theory investigation of the spin interactions for the proposed thiocytosine-centered hole trap has been done. The calculated results are in much better accord with the experimental data if the cytosine molecules surrounding the thiocytosine-centered trapped hole in the lattice are taken into account. The mechanism of hole transfer and trapping is discussed.

9-7

Application of enzyme field-effect transistors for control of quality and maturity of potatoes

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Quantity and ratio of monosacharides and starch in potatoes strongly depend on cultivation and storage conditions. Low glucose content (less than 3 g /1 kg of fresh weight) is an indicator of maturity of potato tubers. For glucose determination in potato juice glucose-sensitive field-effect transistors (ENFETs) with enzymatic membrane were applicated. Glucose concentration in different potato juice samples determined by ENFETs (by using the linear portion of the calibration curve up to 2 mM glucose in a model solution) were compared with those measured by colorimetric method and good correlation between these methods was revealed. The precision of analyze using ENFET didn't depend on nitrates or nitrites content in samples. The high reproducibility of the biosensor developed was demonstrated.

9-8

Fluorimetric biosensor for superoxide radicals

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Superoxide anion radical, one of the reactive oxygen species (ROS), is produced by a variety of cellular mechanisms and has been shown to play an important role in a wide variety of clinical diseases, including heart attack, ischemia and cancer. However, in most cases, the biological significance and mechanism of the generation of superoxide anion is not well understood. Theoretically predicted concentration of superoxide peak is in the submicromolar range. It is thus of great importance to evaluate quantitatively dynamic changes in superoxide concentration using selective detectors with nanomolar detection limits. In this work, we have developed a fluorescent biosensor for superoxide radicals based on the coupled reaction: superoxide dismutase (SOD)-horseradish peroxidase (HRP) enzymes and the probe N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red), with a detection limit at the nanomolar range. Superoxide anion radical was produced via oxidation of xanthine by xanthine oxidase (XO). The enzime SOD catalyzes the dismutation reaction of the generated superoxide radical with the release of hydrogen peroxide, wihch in the presence of HRP reacts stoichiometrically with the non-fluorescent Amplex Red to generate the red-fluorescent oxidation product resorufin. The coupled SOD-HRP system was immobilized in a single sol-gel matrix. Experiments on the stability, activity and capability to reuse the immobilized enzymes are currently under progress. Work supported by grants PI020606 (Instituto de Salud Carlos III, Spain) and MAT200203515 (MCyT, Spain).

Posters
- Biomaterials and bionanotechnology -

9-9

Fluorimetric assay for uric acid detection using coupled uricase-peroxidase system immobilized sol-gel glasses

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The sol-gel method was used to immobilize a coupled uricase/peroxidase system and to develop a highly sensitive ready to use fluorescent biosensor for uric acid detection. The probe Amplex red was used for the fluorometric measurement of peroxide hidrogen in the coupled enzymatic assay allowing the detection of uric acid at very low concentrations. The relative activities of the encapsulated uricase/peroxidase system correlated closely with those of the soluble enzymes illustrating that the sol-gel encapsulation preserved the hierarchy of the enzyme activity. The lower detection limit of the prepared biosensors was found 20 nM and it was 100 times more sensitive than the currently available fluorimetric or colorimetric assays. Reusability of the encapsulated system for various cycles was demonstrated. The possibility to encapsulate simultaneously the enzymatic complex and the fluorescent probe was also checked. Results indicate that the Amplex red encapsulation does not modify the activity of the enzymes and detects, with the same precision, the acid uric concentration of the different samples. The reliability of the prepared biosensor was cheeked by measuring the concentration of uric acid in a number of urine, plasma and blood samples using the fluorescent method and a commercially available uric acid diagnostic kit. Results show that our biosensor allows the determination of uric acid on biological fluids by simple dilution (up to 10000 fold), without any sample pretreatment.

9-10

Studies of plasma blood component adsorption by exfoliated graphite

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It is known many applications of different forms of carbon, including graphite and exfoliated graphite. Adsorption property of carbon is well known also. However, adsorption of protein and other biological molecules by exfoliated graphite is of subject of interest due to possible application. Process of adsorption of plasma blood components by exfoliated graphite has been studied by FTIR spectroscopy, namely, one of the new supersensitive methods - surface enhanced infrared absorption (SEIRA) spectroscopy. We studied different plasma components (cholesterol, albumin, hemoglobin, etc.,) before and after interaction with exfoliated graphite of different content. All samples was precipitated on gold rough substrate and recorded in geometry on reflectance. Conventional adsorbent was used as reference. The SEIRA data showed the following features: i) exfoliated graphite could be used as adsorbent with less efficiency than conventional adsorbent ii) some specific interactions of exfoliated graphite with proteins were observed iii) technology of exfoliated graphite influence the adsorption property.

9-11

Growth and differentiation of osteogenic cells on calcium phosphate glasses

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Materials that augment bone cell proliferation and osteogenic activity have important therapeutic implications for bone regeneration and for use in skeletal reconstruction and joint replacement. We have studied the growth and differentiation of MC3T3-E1 cells on calcium phosphate glasses in vitro. These materials present chemical composition similar to the bone mineral phase. In this study, two different formulations of phosphate glasses in the system P2O5-CaO-Na2O-TiO2 have been assayed. We have evaluated some characteristics about their biocompatibility. MC3T3-E1 pre-osteoblast cells were cultured in direct contact with the glasses in alpha-MEM medium supplemented with 10% foetal bovine serum during 10 days to evaluate the proliferative stage. Then the cell culture were induced to differentiation with ascorbic acid and beta-glycerophosphate as supplement of the culture medium and the culture were prolonged by 27 and 37 days to evaluated the formation of osteoblasts and mineralization stages. In all the materials evaluated the cell culture had a normal proliferation and differentiation rate and it was evidence by the concentrations of DNA, RNA, and total protein. The sodium, calcium and phosphorus in the supernatant of the culture medium were determined in order to evaluate the material biodegradation.

9-12

Self Assembly Stress in a Bipolar Phosphocholine - Water System: Fibrils and Nanoparticles

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The bipolar compound dotriacontan-1,1'-diyl-bis-[2-(trimethylammonio)-ethylphosphat] (PC-C32-PC) in water forms novel types of aggregate structures due to the long alkyl chain and the large polar headgroups at both chain ends a mismatch between polar and non-polar parts. In diluted samples (< 99.5 wt% water) a highly viscous almost transparent gel is observed. At temperatures above 70 °C the gel disappears and a fluid solution is obtained with low surface tension and extreme wetting behavior. DSC measurements revealed different endothermic peaks upon heating and a strong hysteresis in the transition behavior. A peak at 50°C is probably caused by a transition in the chain region. FT-IR measurements confirm this assumption. An attempt was made to characterize the particles at high temperature and in supercooled solutions with DLS measurements. The main fraction of the sample consists of small mono-dispersed nanoparticles. With AFM we detected lamellae, fibrils, or particles in the suspension. Estimates of step heights showed steps corresponding to the length of the molecule (6 nm). With freeze fracture EM parallel stripes were seen as well as mono-disperse nanoparticles. Negative staining and cryo EM of diluted suspensions revealed a network of stiff fibers. It can be concluded that the bola compound cannot form lamellae. Fibrils with rigid chain packing appear at low temperature. At the transition at higher temperature the aggregate size and morphology changes dramatically. Mono-disperse nanoparticles are formed.

Posters

- Biomaterials and bionanotechnology -

9-13

In vitro studies on the interaction of cells with biomaterials based on polypropylene and polydioxanone

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Polypropylene monofilament and multifilament meshes are frequently used for the medical treatment that require the addition of a reinforcing or bridging material to obtain the desired surgical result. It has been observed that the multifilament mesh (PPmulti) elicits initial inflammatory reaction in tissues while the monofilament one (PPmono) is better tolerated by patients. It is known, however, that the mechanical properties of the multifilament meshes are much more satisfactory than monofilament ones. Thus we tried to improve the biocompatibility of PP mesh by covering the mesh with the layer made of polydioxanone (PDS).
The analysis of the IR and SEM data revealed that the degradation of PDS was accelerated in the presence of human macrophages, fibroblasts and osteoblasts. The viability of the cells and the concentration of collagen type I was extremely low in cell cultures with PPmulti and considerably higher in the presence of PPmono. The secretion of collagen was intensified by the composites PP+PDS. SEM studies confirmed the presence of the adhered cells only on the surface of PDS and PPmono+PDS.
It can be concluded that the biocompatibility of polypropylene monofilament mesh is much more satisfying than multifilament one; the biocompatibility can be improved by covering the mesh with a layer of absorbable polymer.

9-14

Bioresorbable macroporous ceramic material

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With a special technology the elaboration of a bioresorbable macroporous ceramic material is attempt, which has a possible utilization in surgical orthopedy or in maxillo – facial surgery. As well elaborating different forms and dimensions some applications are possible in pharmaceutical product preparations, utilize the boiresorbable macroporous ceramic material as support of medicine for a slow continous absorbtion, with a retard effect.
The elaboration of the macroporous bioresorbable ceramic material was realized in two steps. In first stage was prepared a microporous ceramic powder as granule based on tricalcium phosphate (β- TCP), and in second phase the macroporous bioresorbable ceramic material on a sponge supporting. The granule obtained in the first srepp has a 30 – 50 % porosity with 1 – 5 μm pore dimensions, the final macroporous ceramic product with a sponge structure has 70 – 80 % porosity and the pore dimensions are 0.5 – 1 mm and can be varied as function of sponge typ.

9-15

Direct observation of gold nanoparticles spontaneously generated within onion-type multilamellar vesicles by cryo-tem

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Synthesizing inorganic particles within constrained domains such as microemulsions, dendrimers, lamellar lyotropic liquid crystals and liposomes is explored to control particle shapes or sizes, taking benefit of matrix confinement. These organic-inorganic materials can be used to reach specific properties. For instance, there is a strong interest in synthesizing gold particles inside lipidic systems for biological applications. Indeed, gold particles, as electron-dense metal, can be used as probes to trace liposomes-cell interactions. The *in situ* growth of gold nanoparticles has been reported for small unilamellar or multilamellar vesicles. Here, gold nanoparticles were generated without adding any external reducing agent into onion-type multilamellar vesicles. Usually, gold nanoparticles formation is asserted by conventional electron microscopy. However, the above technique requires vesicles drying and staining to enhance the contrast. Such pretreatment leads to formation of electron-dense aggregates (artifacts) that could be mistaken for metallic nanoparticles. In this paper, we use cryogenic temperature-transmission electron microscopy (cryo-TEM) to show embedding of nanoparticles synthesized in between lipidic lamellae. We observe for the first time the coupling between the growth of the particles and the structure of the templating lamellar phase. We also propose a simple mechanism of particles growth within multilamellar vesicles based on cryo-TEM and TEM observations.

Posters
- Ion channels (I)-

10-1

The secretory granule as an intracellular Ca^{2+}/H^{+} ionic oscillator. Potential role in secretion

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The ubiquitous role of Ca^{2+} in intracellular communications has undergone important progress. However, considering the number of Ca^{2+}-sensor/effector molecules present in the cell, the question of how messages are delivered to specific intracellular targets has been constrained to localization, and/or amplitude/duration and frequency encoding of the Ca^{2+} signal. Conditional Boolean arguments like "if" or "and"—requiring more than one signals for effective transmission—still remain vastly unexplored. Although both vesicular Ca^{2+} release and maintenance of granular proton gradient are critical for membrane fusion, the presence of a combined H^{+}/Ca^{2+} signaling system in secretory cells has not been considered. Inositol-1,4,5-trisphosphate released from the plasma membrane signals nearby secretory granules to generate a frequency encoded Ca^{2+} message to receptor proteins in the exocytic site (Nguyen et al., 1998. Nature 395, 908-912; Quesada et al., 2001. Biophys. J. 80, 2133-2139). Here, we show that inositol-1,4,5-trisphosphate can also turn the granules into proton oscillators, producing periodic efflux of H^{+} and corresponding pH oscillations in the perigranular vicinity. This combined H^{+}/Ca^{2+}-signal could considerably enhance the specificity of the information sent by the granule by relaying two frequency encoded messages directed exclusively to proteins like calmodulin, annexin, or syncollin which participate in exocytosis and require specific combinations of Ca^{2+} "and" pH for their action.

10-2

Members of the toll-like / IL-1 receptor family form signaling units with maxik channels in macrophages

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Transmembrane signaling initiated by bacterial virulence factors and by Interleukin (IL)-1, respectively, is mediated by particular members of the Toll-like receptor (TLR)/IL-1 receptor family. Patch-clamp experiments on excised outside-out patches of human monocyte-derived macrophages provided evidence that the potassium ion channel MaxiK is activated by bacterial lipopolysaccharide (LPS). According to these data MaxiK appears to be involved in the initial steps of transmembrane signaling by LPS: Channel activation as well as cytokine production induced by LPS can be inhibited by the specific MaxiK blocker paxilline, by LPS antagonists, and by antibodies against the membrane-bound receptor CD14. These observations propose a functional cooperation of the LPS-receptor TLR4 and the ion channel. Here we show that MaxiK is activated by the TLR2 ligand peptidoglycan (PG) and by IL-1 in excised membranes of human macrophages, proposing a functional cooperation of receptors of the Toll/IL-1 receptor family and MaxiK as a general principle. Cytokine production from macrophages, initiated by a variety of bacterial products as well as by IL-1, is inhibited by paxilline. Thus, a highly specific blocker of MaxiK expressed on macrophages may serve as an immunosupressant drug against sepsis. Such a drug could inhibit the inflammatory response to a variety of microbial products.

10-3

Arachidonic acid mediates m_1-muscarinic-induced inhibition and enhancement of n-type calcium current in sympathetic neurons

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Muscarinic agonists activate a slow signal pathway that uses an unidentified diffusible second messenger to inhibit N-type calcium currents. To characterize this slow pathway and to discover the unidentified diffusible second messenger, whole cell N-type calcium currents were recorded from acutely dissociated neonatal rat superior cervical ganglion (SCG) neurons using 0.1 mM BATPA as the internal calcium chelator and 20 mM barium as the charge carrier. We found that the muscarinic agonist oxotremorine-M not only inhibits N-type calcium currents at positive test potentials, but also enhances N-type calcium currents at negative test potentials. Furthermore, both enhancement and inhibition were observed in cell–attached patches. Though inhibition has been observed previously, these findings are the first report of a classical transmitter enhancing N-type calcium current. The actions of oxotremorine-M mimic modulation observed with application of arachidonic acid (AA), suggesting involvement of an AA signaling pathway. Moreover, pharmacologically blocking phospholipase C, phospholipase A2 or AA, but not AA metabolism minimized muscarinic modulation of N-type calcium currents, indicating these molecules participate in the slow pathway and that AA may be the unidentified diffusible second messenger. Lastly, we confirmed that the G protein Gq participates in this pathway by blocking muscarinic modulation of calcium currents with anti-Gqα antibody. Our findings in the SCG together with those of previous CNS studies, implicate AA and the slow pathway as a major signaling mechanism used by Gq-coupled receptors to mediate transmitter-induced modulation of calcium channel

10-4

Protein kinase c participates in recovery from trh-induced Erg K^{+} current inhibition in GH3 rat anterior pituitary cells

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The biochemical pathway leading to ERG potassium channel inhibition by the TRH receptor was explored in situ using perforated patch-clamped GH3 pituitary cells and pharmacological approaches. As previously shown, the extent of the TRH effect on ERG currents was not modified by inhibitors of protein kinase C (PKC), protein kinase A or Ca/CaM kinase. A similar result was presently obtained with Rho-kinase inhibitors Y-27632 and HA-1077. Surprisingly, the inhibitory effect of TRH became irreversible in the presence of HA-1077, but only at concentrations at which it is able to reduce PKC activity. The hormonal effect also became irreversible in cells in which PKC activity was selectively impaired with GF109203X, Go6976 or long-term incubation with phorbol esters. Furthermore, the reversal of the TRH effects, but not its ability to suppress ERG currents, was blocked if diacylglycerol generation was prevented by blocking phospholipase C activity with U-73122. The pharmacological profile identified a classical alpha/betaII PKC subtype as the isoform involved in reversion of the TRH effect. Since the hormonal effects also became irreversible after inhibition of protein phosphatase 2A (Barros et al. Pflugers Arch. 422:31-39, 1992; Barros et al. FEBS Lett. 336:433-439, 1993), our results indicate that reversal of TRH-induced ERG inhibition involves a pathway in which protein phosphatase 2A acts downstream of an alpha/betaII cPKC that is activated by phospholipase C-induced diacylglycerol production. If phosphate removal is exerted on the channel itself or from a regulatory component associated to it remains to be established.

Posters
- Ion channels (I)-

10-5

Calmodulin mediates calcium modulation of m-type K^+ channels

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To quantify modulation of KCNQ2/3 current by intracellular calcium (CAi) and to test if calmodulin (CaM) mediates this action, simultaneous whole-cell recording and Ca-imaging was performed on CHO cells expressing KCNQ2/3 channels, either alone, or together with wild-type (wt), or dominant-negative (DN) CaM. We varied CAi from <10 to >400 nM with ionomycin and 2 mM Ca or EGTA-buffered Ca-free solutions. Wt CaM made KCNQ2/3 currents highly sensitive to CAi (IC50 70 nM). However, DN CaM rendered KCNQ2/3 currents largely CAi-insensitive. In cells without co-transfected CaM, Cai sensitivity was weak. CAi modulation of M current in superior cervical ganglion (SCG) neurons followed the same pattern as CHO cells overexpressing KCNQ2/3 and wt CaM. Co-immunoprecipitations showed binding of CaM to KCNQ2-5 that was similar in the presence of 5 mM Ca or 5 mM EGTA. Gel-shifts suggested Ca-dependent CaM binding to an "IQ-like" motif in the carboxy-terminus of KCNQ2 and KCNQ3. We tested whether bradykinin modulation of M current uses CaM. Wt or DN CaM was exogenously expressed in SCG neurons using pseudovirions. With EGFP only, the inhibition was 76%; with DN CaM, it was 31%, and with wt CaM, it was 33%. In all three groups, muscarinic inhibition of M current was normal. We observed similar CAi rises by bradykinin in the three groups, indicating that CaM did not affect Ca release from stores. We conclude that M-type currents are highly sensitive to CAi and that CaM acts as their Ca sensor.

10-6

Effects of an alkaloid from flustra foliacea on recombinant human neuronal nicotinic acetylcholine receptors

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The effects of diformylflustrabromine A (DFF-A), an alkaloid obtained from the briozoan *Flustra foliacea*, on different types of recombinant human nicotinic acetylcholine receptors have been studied. Both homomeric (α_7) and heteromeric ($\alpha_4\beta_2$, $\alpha_3\beta_4$) subtypes, expressed in *Xenopus laevis* oocytes, have been investigated. Ionic currents elicited upon application of pulses of acetylcholine were measured using double electrode voltage clamp recordings. Pre- and co-application of DFF-A with 100 μM acetylcholine produced increases in the peak current in $\alpha_4\beta_2$ receptors in a concentration-dependent manner. This effect was not seen in the other receptors studied. In $\alpha_4\beta_2$ receptors, acetylcholine concentration-response curves in the presence of co-application of DFF-A were slightly shifted toward lower concentrations, while macroscopic current-voltage relationships did not show any change in the reversal potential. In addition, we investigated whether the observed increase in the macroscopic peak current was due either to an increase in the single channel conductance or to an increase in the open probability of the receptor by doing single channel recordings in the cell-attached mode with low concentrations of acetylcholine. The results suggest that DFF-A acts as a specific positive allosteric modulator of human $\alpha_4\beta_2$ nicotinic receptors.

10-7

Dissection of the mitochondrial inner membrane translocases: effects on the channel properties and functioning

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Distribution of proteins within cellular organelles is mediated by specific apparatus that sequentially recognize, sort, transport and release a functional mature protein. Two main pathways for protein import into mitochondria have been described, and three different translocases, one in the outer membrane (TOM complex) and two in the inner membrane of mitochondria (TIM23 complex and TIM22 complex), account for such function. With the aim of defining the mechanism and regulation of the actual protein import channels of mitochondria, a combination of patch-clamping, genetic, and biochemical techniques are used to modify the structure and, hence, function of the import apparatus. Mitochondria from Sacharomyces cerevisiae with reduced expression of Tim23, Tim17, Tim44, or Tim22 have been characterized with this approach. The single channel behaviour, as well as the sensitivity of the channels to signal and non signal sequence peptides (i.e. yCOXIV, P2, SynB2), are used to evaluate the effects of genetic mutation in the protein components of the import complexes. Such dissection of the individual constituents of the protein translocases has allowed us to assign a more defined role for each of them (e.g. essential, receptor, pore) in the overall functioning of the complexes. Significantly, Tim23 act as the receptor, Tim 17 is essential for normal channel gating and both of them are essential for pore formation. Our studies also confirm Tim44 pulls the protein across the channel and Tim22 does not alter the normal functioning of TIM23 complex. (Supported: Grants Junta Extremadura 2PR02B007, DGICYT PB980988, NSF MCB-0096206, CAPES-047019 fellowship to PMVP).

Posters

- Biophysics bioengineering of sensory system -

11-1

Computational determination of refractive index distribution in the crystalline cones of the Euphausia superba eye

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Euphausiaceae are a family of shrimp-like animals ("krill"), which abound in the dim conditions of the upper deep sea, and represent a major part of the diet of the great whales. Krill organisms have well developed superposition-eyes, which, as behavioural observations have shown, give them excellent vision. In Euphausia superba the eye-surface is covered by a corneal-layer; crystalline-cones are present directly underneath. Below the cones, which are the major refractile optical elements of the eye, an extensive clear-zone, devoid of pigment, exists. The photoreceptive-layer is located on the proximal side of the clear-zone and light that enters the hemispherical eye through many facets gets focused on this layer. In order to understand more accurately how the eye of E. superba functions, one needs to know the distribution of the refractive index (N) in the crystalline-cones. This however, is not available from fixed material. Morphological parameters (curvatures, distances, etc.) of the eye of Euphausia, were obtainable by light microscopy from fixed material. The N of various different tissues (with the exception of the crystalline-cones) of the Euphausia eye were known or could be estimated. With those data, we employed a computer model to determine the "most realistic" spatial distribution of N in the crystalline-cone that would enable the eye to produce a sharp image on the retina. The result of our calculation has to be seen as a prediction of the real situation and it would be interesting if future measurements on crystalline-cone of the superposition-eye of Euphausia could confirm it.

11-2

Biomimetic lipid/polymer colorimetric membranes: molecular and cooperative properties

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Characterization of membranes and of biological processes occurring within membranes is essential for understanding fundamental cellular behavior. Here we present a detailed biophysical study of a recently developed colorimetric biomimetic membrane assembly constructed from physiological lipid molecules and conjugated polydiacetylene. Various analytical techniques have been applied to characterize the organization of the lipid components in the chromatic vesicles and their contributions to the observed blue-to-red color transitions. Experiments reveal that both the polymerized units as well as the lipids exhibit microscopic phases and form domains whose properties and bilayer organization are interdependent. These domains are interspersed within mixed lipid/polymer vesicles that have a size distribution different from those of aggregates of the individual molecular constituents. The finding that fluidity changes induced within the lipid domains are correlated with the chromatic transitions demonstrates that the colorimetric platform can be used to evaluate the effects of individual molecular components, such as negatively charged lipids and cholesterol, upon membrane fluidity and thermal stability.

11-3

Electrophysiological identification of taste cells

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A population of taste receptor cells (TRCs) is heterogeneous and several different cell subclasses have been identified morphologically. Their roles in taste bud physiology are debatable though evidence suggests distinctive cell subtypes to be chemoreceptive. In electrophysiological experiments, TRCs may not be identified unambiguously based on their morphology, so the main goal of the present study was to elaborate electrophysiological criteria for the identification of TRCs. We recorded from isolated mouse TRCs and found that those can be subdivided into three distinct groups on the basis of their electrical characteristics. Most of TRCs showed VG Na+ currents. However, this cell subpopulation comprised two different subgroups being distinguishable by the magnitude of VG Na+ currents, kinetics and magnitude of VG K+ currents and tail currents. (group A and group B). A distinctive TRCs exhibited no VG Na+ currents and peculiar VG K+ currents (group C). To elucidate whether TRCs expressing gustducin, a taste specific G-protein, may be assigned to any particular subpopulation identified electrophysiologically, a number of experiments were performed using transgenic mice, gustducin positive TRCs of which express green fluorescent protein (GFP). We successfully recorded from gustducin positive TRCs identified by their green fluorescence and all of them were found to belong to the group A. Thus, the electrophysiological criteria may be applicable for the identification of TRCs.

11-4

Modeling retinal mosaic formation by local machanical interactions

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The retina is one of the best modular organization examples in neural circuitry. This modular structure enables the retina to perform parallel processing. The retinal neurons are placed in various layers in the retinal tissue. Within every layer, neurons of the same type organize themself in an ordered way, forming the retinal mosaic. We have set up a mathematical model of retina focusing on the mechanical features of retinal neurons and on the interaction and dendritic overlapping among neurons. The model is based on the assumption that interactions among neurons are local and mechanical. These mechanisms allow each retinal cell to exclude that cells of the same type can be closer than a fixed minimal distance, which is typical of the cell type, and that the spatial range of the interactions between omotypic cells depends on the neuron's type. We have used a model of cytoskeleton based on the tensegrity concept, that is the cytoskeleton is regarded as a structure composed by elastic and rigid elements. We have assumed that neurons repel each other in proportion to the degree of the dendritic overlap; these overlaps depend on the growth of the dendrities due to the cytoskeletric deformation. The results obtained are in agreement with experimental results showing that small cell movements, due to local mechanical interaction and dendritic overlapping, are able to transform random cell distributions into regular mosaics.

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Posters

- Biophysics bioengineering of sensory system -

11-5

The influence of the neuroleptic treatment on the fractal dimension on the eyeball movement in patients with diagnosed schizophrenia

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Many authors describe differences in tracking and directing movements of eyeballs in patients with a diagnosed schizophrenia in comparison with the control group. Human eye, besides the tracking and directing movements, has also micromovements with very low amplitudes and high frequencies. In our opinion those movements are responsible for the dissimilarity of schizophrenics' eyes. In our earlier papers the method of differentiation of the micromovements of the human eyeball was presented. The picture of the eyeball was obtained with the help of the slit lamp with a built-in video camera. The subsequent frames were stored in computer's memory. In this way the software allowing to determine the position of the centre of the pupil was created. As a result, we obtained a set of points on the plane showing the position of the pupil on the subsequent frames. For this set of points the fractal box dimension was calculated. In a group of patients with a diagnosed schizophrenia and receiving neuroleptic treatment (CLOZAPINE, OLANZAPINE, RISPERIDONE, CLOPIXOLDEPOT - 20 people) the value of box dimension was markedly different than that in a control group. This phenomenon, however, not described in literature on schizophrenia, deserves further scientific verification as a potential marker for neurophysiological disturbances connected with schizophrenia

11-6

Improving polypyrrole glucose oxidase biosensor by encapsulation of the enzime in multilamellar vesicles

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Biosensors may use enzymes as catalysts to detect and measure a substrate concentration either in a natural medium (blood, seawater, …) or not (foodstuff, beverages, …). Enzyme immobilization is required to produce reusable and low cost analytical biosensors. In that aim, a well known technique consists in entrapping the enzyme in a redox polymer grown on the surface of an electrode and therefore the reaction with the substrate can be electrochemically monitorized [1]. However, this technique presents major drawbacks : the substrate concentration linear response range of the biosensor is not high enough for concentrated media while the signal intensity is low and so the biosensor sensitivity. In order to improve the biosensor efficiency we have encapsulated an enzyme, the glucose-oxidase, into multilamellar vesicles, the so called onions [2,3], prior to the electroformation of the polypyrrole flm. We show a 100 fold increase in the signal compared with the conventional method and a higher linear range. We have characterized the film growth in presence of the vesicles and compared the efficiency of the glucose-oxidase biosensor formed with or without onions as a function of the film thickness, the pyrrole concentration, etc.

[1] M. Shin, H. Kim, *Biosensors and Bioelectronics* 11 (1996) 171
[2] O. Diat, D. Roux, *J. Phys II France* 3 (1993) 9
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11-7

Spectroscopic characterization of the pigment responsible for the behavioral responses to UV-blue light in a mutant of halobacterium salinarum devoid

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The mutant Pho81W of Halobacterium salinarum is devoid of rhodopsinic pigments but it still responds to blue light of high intensity by reversing the swimming direction. The action spectrum of these photophobic responses is reported starting from 360 nm and suggests that the involved pigment is actually specific for UV lights. To characterize spectroscopically this pigment we performed flash-photolysis measurements on vesicles suspensions obtained by sonication from Pho81W cultures. By using a UV enhanced Xenon lamp as excitation light we measured the excitation spectrum starting from 320 to 450 nm. We carried on two kinds of measurements. In the first one we selected the excitation light by using narrow band inteference filters and measured the transmitted light at a fixed wavelenght of the analysis light, so getting the shape of the absorption spectrum in the ground state. In the second approach we used a fixed wavelenght for the stimulating light and measured the transmitted light at various wavelenghts of the analysis light, so getting the shape of the absorption of a long lived intermediate. We thank John ed Elena Spudich (Medical School, University of Texas, Houston) for the Pho81W mutant.

11-8

Multilifetime and Multicolor Colocalization of Single Molecules

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We present a technique for ultrahigh-resolution colocalization of conventional single fluorescent dyes with nanometer accuracy using multi-lifetime, and multi-color detection, i.e. Spectrally-resolved Fluorescence Lifetime Imaging Microscopy (SFLIM). The method takes advantage of single fluorescent dyes that can be efficiently excited by a single pulsed diode laser emitting at 635 nm but differ in their emission maxima, and in their fluorescence lifetime. A combined analysis of the fractional intensities and fluorescence lifetimes recorded on two spectrally-separated detectors enables the classification of the portion of each dye per pixel in a point-spread-function (PSF) image with high accuracy, even though only a limited number (generally a few thousand) photons are detected per single dye. From these portions, two separate PSF images are calculated and fitted to two-dimensional (2D) Gaussian functions. As a scaffold to keep two dye molecules at a well-defined distance, we use end-labeled DNA of different lengths. With the DNA-model system, we show that two molecules can be localized with a precision of a few nanometers. To circumvent patchy PSF images and subsequent lower precision in localization, the off times of the dyes used (triplet lifetime) should be shorter than the integration time per pixel. Furthermore, the dyes should exhibit constant fluorescence emission maxima and lifetimes. We demonstrate that by the use of appropriately selected dyes, the presented technique permits the determination of the distance between two single dye molecules in the nanometer range with a precision of ca. 10 nm without any chromatic abberations.

Posters

- Biophysics bioengineering of sensory system -

11-9

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 4-hydroxy-cinnamic acid chromophore. This initiates a large conformational transition, leading to a phototactic response of the bacterium. Photoactivation initiates a photocycle ($\Phi = 0.35$) 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 light-induced deprotonation of its FAD chromophore, forming a signaling state which recovers with a rate of $\sim 10^{-3}$ s^{-1}. Furthermore, also this photoreceptor is partially unfolded in its signaling state. It is a challenge to resolve the role of these partially unfolded signaling states for biological signal transfer.

Posters

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

12-1

Interactive effect of boron and salinity stress on water transport through membranes of root cortical cells

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Proteins which form water-channels or 'aquaporins' in membranes have been identified in plants. Water-channels allow water to pass freely across the cellular membranes, following osmotic or hydrostatic pressure gradients. Water-channels in tissues are known to be blocked by sulphydryl reagents such as mercurials, e.g. HgCl2. In our previous experiments, we have suggested that NaCl could decrease water-channel activity by affecting the protein. Also, it has been proposed that boron, when supplied at normal soil concentration, can be transported by aquaporins or other Hg-sensitive channels. In this work we have studied the effect of NaCl and B on cell water transport though aquaporins in Zea mays L. amylacea, which grows naturally in soils with high contents of NaCl and B. Plants were grown in hydroponic culture with modified Hoagland nutrient solution, in a growth chamber. Cell hydraulic conductivity of root cortical cells was measured with a cell pressure probe, by changing hydrostatic pressure inside cells, in plants treated with 100 mM NaCl and/or 1.8 mM H3BO3, before and after treatment with Hg. The root cortical cells showed a decrease in hydraulic conductivity, Lpc, when plants were treated with NaCl, but Lpc remained constant with B treatment. Interaction between the two treatments produced a decrease in Lpc similar to that obtained for NaCl-treated plants. Mercuric chloride treatments only reduced Lpc in control and B-treated plants. These results may indicate that if NaCl is reducing the functionality of aquaporins, water and boron transport through membranes could be decreased greatly.

12-2

Aquaporin 6 (AQP6) is permeable to glycerol and urea

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AQP6 is located mainly to the membranes of intracellular vesicles but its function is not yet precisely defined. In contrast to other AQPs, its water permeability (Lp) is activated by sub-millimolar concentrations of Hg2+ and it conducts anions at external pHs below 5.5. Our aim was to test the permeability of AQP6 to small hydrophilic molecules. Xenopus laevis oocytes expressing AQP6 were placed in a chamber in which the bathing solution could be changed within 5 sec and the oocyte volume monitored with an accuracy of 0.03% (0.3 nL). With 0.3 mM of HgCl2 in the bathing solution the oocyte began to swell about 0.3%/min. The Lp of the AQP6 was determined from osmotic challenges (60 sec) obtained by adding (or removing) mannitol, glycerol or urea. The Lp(mannitol) was 2.4 +/- 0.1 (14 oocytes), Lp(glycerol) 1.5 +/- 0.2, and Lp(urea) 0.7 +/- 0.1 (units: 10-5 cm sec-1 Osm-1), the latter was not different from that of native oocytes. The Lps were independent of the degree of HgCl2 induced swelling and of the magnitude of the osmotic challenge (-75 to +100 mOsm). The presence of AQP6 increased the uptake of [14C]glycerol and [14C]urea by a factor of 5 and 2 respectively above that of native oocytes or AQP1 expressing oocytes whether these were treated with HgCl2 or not. Our data suggest that water, glycerol and urea share an aqueous pathway in AQP6 and that AQP6 belongs to the group of aquaglyceroporins.

12-3

A combined study of the structure and dynamics of water around lysozyme using neutron crystallography/spectroscopy.

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Cells are highly macromolecular-crowded media, and a precise knowledge of the intracellular water properties is necessary in order to understand various biological mechanisms. This work reports a combined study of the structure and dynamics of water in triclinic crystals of lysozyme. A model of the water behaviour under a few hundred ps, at 300 K and atomic resolution, is proposed. As protein crystals have a highly crowded arrangement, this model can be discussed in the light of solvent behaviour in cells.
Neutron diffraction data were collected at 1.7 A resolution [1]. A model with all hydrogen atoms on the protein, and 244 out of the 310 water molecules theoretically present in the unit cell was constructed. Interesting characteristics about the disorder of the interfacial water, the structure of water around apolar parts of the protein were observed.
The single-particle diffusive dynamics study was done on protein crystals using neutron spectroscopy [2]. It showed that all water molecules have their dynamics affected by the presence of the protein. Two populations were observed. One in which water molecules reorient themselves 5 to 10 fold slower than in bulk solvent, and jump from hydration site to hydration site, with a long-range diffusion coefficient reduced 5 fold compared to bulk solvent. The second group of water molecules undergo a long-range translation, with a diffusion coefficient reduced about 50-fold compared to bulk solvent.

12-4

Experimental evidence for water transport in NaKCL cotransporters

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The NaKCl cotransporter isoform NKCC1, ubiquitous in secretory cells, was studied in cultures from the pigmented ciliary epithelium of the human eye (PE cells). Another isoform, NKCC2, from the water impermeable thick ascending limb of Henles loop, was studied in cultures from the rabbit (TALH cells). The comparability of the two cultures was ascertained by electron microscopy and antibodies to NKCC1 and NKCC2. Transport rates, determined from 86Rb-uptake, were about 30% higher in TALH than in PE cells. Water transport was studied by fluorescence self-quenching of Calcein. The osmotic water permeability Pf of PE cells was 85 +/- 5 microm/sec (n=89) and the reflection coefficient for NaCl 0.53 +/- 0.04 (n=26). Pf was inhibited 48 +/- 3 % (n=7) and the reflection coefficient increased to 0.98 +/- 0.07 (n=4) by the specific inhibitor bumetanide (10 micromol/l). In TALH cells Pf was about 3 times smaller, and the reflection coefficient for NaCl equalled one; bumetanide had no effects. In PE cells replacement of Cl by inert anions caused rapid cell shrinkage (1.4 +/- 0.1 %/sec (n=52)), while re-addition of Cl caused rapid re-swelling (1.3 +/- 0.1 %/sec (n=49)). In TALH cells both effects were more than 4 times slower. In PE cells this re-swelling could proceed against an osmotic gradient of more than 50 mosm/l of mannitol; the shrinkage and re-swelling were inhibited by bumetanide and removal of Na. We conclude that NKCC1 transports water actively while NKCC2 does not.

Posters

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

12-5

Hypercapnic chemotransduction in the rat adrenal medulla. role of AQP-1.

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In this work we have studied the participation of adrenal medullary (AM) cells in hypercapnic chemotransduction and the possible role of aquaporin-1 (AQP1) in this process. Rat neonatal AM cells are known to be O2-sensitive during a period (from P1 to ~P15) at which the carotid body is still immature. Therefore, we investigated whether AM cells are also physiologic CO2 chemoreceptors. In addition, we have tested the hypothesis that the water channel AQP1, postulated to be permeant to CO2 in some systems, contributes to facilitate the CO2 responsiveness in AM cells. Here we show results indicating that AM cells are able to sense hypercapnia. Using amperometric recordings we have measured catecholamine secretion from cells in AM slices from neonatal (1-8 days old) and adult (>= 1 month old) rats, perfused with solutions bubbled with different concentrations of CO2 at constant pH (7.4) and O2 tension (150 mm Hg). The secretory activity (in fC/min, mean ±se, n=7) increased from 3532 ± 904 with 5% CO2 to 7063 ± 2094 and 18110 ± 6877 with 10% and 20% CO2, respectively. Responsiveness to hypercapnia was more frequent in neonatal slices (64 % of cells tested, n=61) than in the adult (35 %, n=17). The secretory response to hypercapnia required extracellular Ca++ influx, as it was abolished by extracellular Cd++ (0.5 mM). Supporting the potential role of AQP1 in this process we have shown the presence of AQP1 and carbonic anhydrase in AM cells by RT-PCR and in situ hybridization. Interestingly, the time course of AQP1 expression in the AM showed a peak around 1-8 days after birth, consistent with the increase in the secretory response to CO2 at that age. Thus, our data show that AM cells are CO2 sensors, particularly during neonatal life, and support the ould have a participation in this process.

12-6

Field measurements of diurnal diameter changes: Supporting Munch hypothesis

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Tree stem diameter varies diurnally due to changes in the water content of the stem. This variation has been detected since the late 19th century and its connection to various environmental factors has been reported. However, most of the measurements have been done on the whole stem (i.e. on bark) and the different function of xylem and phloem in the sense of water transport has not been taken into account. The connection of xylem diameter variations to transpiration and the ascent of sap has been conformed quite recently both experimentally(1) and theoretically(2) and the results support the cohesion theory. We have measured xylem and stem diameter variations on Scots pine (Pinus sylvestris L.) trees at SMEAR II station in Hyytiala, Southern Finland and compared the variations of xylem and whole stem at different heights. The variations of the whole stem do not follow transpiration as thoroughly as xylem diameter variations. Comparing the timing of the variations with the predictions of Munch hypothesis of the mechanism of sugar transport suggests that phloem flow and phloem-xylem interactions could be detected, even in field conditions, using this kind of measurement set-up. (1) Irvine J. and Grace J. (1997) Continuous measurement of water tensions in the xylem of trees based on the elastic properties of wood. Planta 202:455-461. (2) Peramaki M., Nikinmaa E., Sevanto S., Ilvesniemi H., Siivola E., Hari P. and Vesala T. (2001) Tree stem diameter variations and transpiration in Scots pine: an analysis using a dynamic sap flow model. Tree Physiology 21:889-897.

12-7

Studies on Water Transport and CFTR channel in membrane vesicles from two different cell lines

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The influence of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) on water movement across cell membranes has been reported. To further establish this correlation osmotic water permeability studies were preformed in plasma membrane vesicles prepared from different cell lines that proved to be useful models in the study of chloride channels by patch clamp techniques: Caco2 a cell line that normally express the CFTR channel was used as positive control while CHO cells that normally do not express the CFTR channel were used as the negative control. CHO transfected with the wild type CFTR gene was then used to see if the water permeability was in any way different from the CHO control. Membrane vesicle preparations were obtained from culturing the cells in appropriate medium and subjecting them to cell disruption in order to obtain isolated plasma membranes; the purity of these membrane vesicles was assessed by evaluating the enzyme markers activity; the vesicular volume was measured by dynamic light scatter (DLS) and the water flux across the vesicles followed with the stop flow technique. The activation energy (Ea) was measured for these different vesicle populations under different experimental conditions, which include the use of CFTR activators (cAMP, ATP, IBMX). Ea was found to be high for all the preparations tested, and these Ea values were not different even when activators were present in the incubation media. The results did not show any correlation between the activity of the CFTR channel and the water transport in membrane vesicle preparations.

12-8

Water mass concentration local measurement on hydrated biological cryosections by eels in spectrum-imaging mode.

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In biological cells, water is an important component which is in strong interaction with macromolecules and operates in many biochemical processes. Water is also closely related to ionic fluxes and mechanisms of cellular volume regulation. Then the water concentration measurement can allow to study cellular life phenomena and some pathologies. We have developed a direct water concentration measurement method using electron energy loss spectroscopy. We record energy loss spectra over an area of an hydrated cryosection in the spectrum-imaging mode. The obtained lateral resolution is approximately 200 nm. We developed a software to process data in order to get water content map in the studied area. The quantification requires the use of two reference spectra: the single scattering distributions of the amorphous ice and of a protein, the bovine serum albumin (BSA). By comparing a weighted sum of these reference spectra to the single scattering distribution of the experimental spectrum, we obtain fitting coefficients corresponding respectively to water and macromolecular compounds contributions of the studied sample. The water mass content is then determined at each point of the analysed area. We can then build up an image in which grey levels are proportional to the water content. In this image, we can recognise the different cellular compartments and we can average the water content in each of these compartments. This method can be applied to the study of water mass content variations in cryosections of biological cells. It gives some important informations on cellular alterations or modifications like apoptosis, exocytosis.

Posters

- Membrane Structure and Dynamics -

13-1

Effects of unsaturated free fatty acids and triglycerides on the structural properties of phosphatidylethanolamine membranes

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Membrane lipid composition can be modulated to balance the contents of pro- lamellar- and nonlamellar-lipids to regulate its structural properties and their functions. It is known that the composition of some biological membranes is influenced by dietary fats. For example, diets rich in oleic acid are associated with increases in the levels of oleic acid in various plasma membrane in rats and human. It has been reported that biomembranes can incorporate fatty acids and triglycerides into the bilayer structure (Hamilton J. A., et al. J. Lipid Res. 37 (1996) 773-782; Langner M., et al. Biochim. Biophys. Acta. 1236 (1995) 73-80). However, the molecular mechanisms involved are not fully understood. This study focus the interest on the effect of the unsaturation degree of the C-18 analogues, oleic, linoleic and linolenic acids, and their triglycerides, triolein, trilinolein and trilinolenin, on the structural properties of lamellar and nonlamellar lipids. By X-ray diffraction and 31P-NMR analysis, both kind of molecules appeared to alter the thermotropic behaviour of phosphoethanolamine liposomes, althought they showed a differential efficiency on the perturbation of the lipid phases. This effect is explained on the basis on their molecular shape. The fluorescence polarization data added information on the effect of oleic acid and triolein on the lamellar Lalpha and nonlamellar HII phases properties.

13-2

On the study of the effect of lamellar- and nonlamellar-forming lipids with synthetic peptides of G-protein and alpha2 adrenergic-receptor sequences

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Membrane lipid structure plays an important role in various cell functions, such as the modulation of signal transduction and membrane protein activity. The interaction between protein and lipids is crucial for their functional properties. Two important questions, not yet resolved, are: (1) the significance of the lipid composition on the structural lamellar-nonlamellar membrane domains and (2) the mechanisms by which the biological membrane function could be modulated. We are interested to ascertain the role of the membrane in the G protein-coupled receptor signal transduction. Some roles attributed to hexagonal (HII)-prone phospholipids include the regulation of G protein and protein kinase C localization and activity (Escriba, P. V., et al. 1997. Proc. Natl. Acad. Sci. USA. 94: 11375-11380). To get a detailed understanding of protein-lipid interactions and how the membrane composition regulates the signal transduction through G protein-coupled receptors, we investigated the behaviour of peptides corresponding to G protein subunits and transmembrane domains of G protein-coupled receptors, on the lamellar or nonlamellar lipid phase stabilization. The aims of this study are to assess: (1) the interaction of synthesized peptides with lamellar or nonlamellar lipid-phases and their phase behaviour and (2) the effect of the fatty acids on the lipid-peptide interaction. X-ray diffraction and fluorescence measurements showed that: (i) G protein peptides interact with lamellar structures, and (ii) transmembrane domains of G protein-coupled receptor peptides can alter the phase behaviour of phosphoethanolamine membranes, giving information about the structural properties of lamellar and nonlamellar

13-3

Thermal transitions of glycolipids from brown alga sargassum pallidum

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Thermotropic behavior of unhydrous glycolipids, isolated from marine alga Sargassum pallidum was studied by differential scanning calorimetry and polarizing microscopy. Fatty acids of lipids was analyzed to interpreted their thermal transitions. It was shown that thermograms of membrane glycolipidsmonogalactosyldiacylglycerol and sulfoquinovosyldiacyglycerol located at temperatures below zero. Phase transition of digalactosyldiacylglycerol was ranged from minus 100 to 74 degrees C. Thermograms of monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacyglycerol were characterized by the main endothermic peak at minus 80, minus 74 and minus 20 degrees C, respectively. The low enthalpy peak located at the high-temperature range between 30 and 60 degrees C on thermogram of digalactosyldiacylglycerol. Using polazing microscopy,it was observed that this low enthalpy transition corresponded with either isotropic melting or mesophase transformation of lipid. The market phase changes at temperatures above zero probably underlie the observed infringements of photosynthetic and mitochondrial activities of marine macrophytes at the sharp increase of seawater temperature up to 30-40 degrees C.The lowest phase transition temperatures of monogalactosyldiacylglycerol and digalactosyldiacylglycerol correlated with the highest level of arachidonic and eicosapentaenic acids and correspondently highest unsaturation index. On the contrary, the highest transition temperature of sulfoquinovosyldiacyglycerol connected with the highest percentage of saturated acids and the lowest unsaturation index. Thus, the absence of effective compensatory mechanism lipid viscosity may be the reason of poor adaptive capacity of poikilotherms to superoptimal temperatures of habitat in contrast to low-temperature adaptation.

13-4

Effect of de novo designed peptides interacting with lipid membrane interface on stability of cubic phases of monoolein membrane

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Elucidation of the stability of cubic phases is essential for understanding of dynamics of biomembranes. Recently, we have found that electrostatic interactions due to surface charges of lipid membranes induce phase transition between cubic phase and La (L alpha) phase and also that between different cubic phases (BBA, 1461, 96, 1999; Biophys. J., 81, 983, 2001). In this report, we investigated effect of a de novo designed peptide (WLFLLKKK) on the stability of the cubic phase of a monoolein (MO) membrane by small-angle X-ray scattering. The peptide-1 has positive charges and a site partitioned into the electrically neutral lipid membrane interface (Langmuir, 18, 9638, 2002). As increasing peptide-1 concentration, a phase transition from Q224 to Q229 phase in the MO membrane at 30 wt% lipid concentration occurred at R = 0.0090 (R is a molar ratio of peptide to MO), and at and above R = 0.040, MO/peptide-1 membranes were in La phase. High concentrations of NaCl in the bulk phase inhibited these phase transitions. These results indicate that the peptide-1 was partitioned into the membrane interface of the MO membrane and the electrostatic interactions due to the peptide-1 in the membrane interface make the Q229 phase more stable than the Q224 phase, and that at larger electrostatic interactions the La phase is more stable than these cubic phases. The mechanism of the effect of peptide-1 on the phase stability of MO membranes is discussed.

Posters

- Membrane Structure and Dynamics -

13-5

Thermotropic behavior of major phospholipids from muscle tissue of marine fish

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It was studied lipid and fatty acids composition as well as thermotropic behavior of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from muscle tissue of Pacific cod (Gadus macrocephalus),banded Irish lord (Hemilepidotus gilberti), Pacificic halibut (Hippoglossus stenolepis), Korean flounder (Glyptocephalus stelleri), whip sculpin (Gymnocanthus intermedius), collected at the dept of 50-90 m at about 3 degrees C. Total phospholipid content varied from 32 up to 68% of total lipids. The content of major phospholipids: PC and PE, reached 90% of total phospholipids. Molar ratio between cholesterol and phospholipids was about of 0.2. By differential scanning calorimetry, it was shown that phase transition of unhydrous phospholipids were ranged from -62 to -44 degrees C up to 20-64 degrees C. The temperature ranges of thermal transitions of both phospholipids overlapped. Thermograms of PC were characterized by the main endothermic peak at -15 degrees C (halibut), -4 degrees C (cod, Irish lord) and 5 degrees C (flounder, sculpin). On thermograms of PE, several lov-enthalpy peaks were revealed at more widely temperature range (-90 to 64 degrees C). Peak maximum temperatures were at -22 degrees C (cod),-10 degrees C (flounder, sculpin), -6 degrees C (Irish lord), 24 degrees C (halibut). The enthalpy of phase transitions of PE was lees, than of PC. Observed differences between thermotropic behavior of PC and PE seem to be connected with distinctions in their fatty acids compositions. Complex calorimetric profile of thermal transitions is proposed to be induced by domain formation into studies lipid samples.

13-6

The hydration effect on thermotropic behavior of glycolipids isolated from marine macrophytes

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Differential scanning calorimetry were used to investigate the character of changes in thermotropic behavior of major lipids of photosynthesizing membranes - monogalactosyldyacylglycerol, digactosyldiacylglycerol and sulfoquinovosyldiacylglycerol, isolated from brown alga Laminaria japonica and seagrass Zostera marina, under hydration in presence or without ethylene glycol in comparison with dry samples. Fatty acid composition of glycolipids was analyzed by gas-liquid chromatography. It was shown the appearance of only one sharp endothermic peak under heating of hydrated glycolipids instead of complex calorimetric profile of low-enthalpy thermal transition revealed by correspondent dry lipids. Hydration induced the shift of peak maximum temperatures in direction to ice melting temperature and promoted much more co-operativity of thermal transitions in comparison with dry samples. More complex thermogram profiles, recorded under cooling of glycolipids, were characterized by low-enthalpy peaks in range of -45 degrees C besides sharp peak at higher temperature range: from -12 to -28 degrees C, that seems to correspond to different lipid domains. That was provided by revealed two peaks on thermograms of glycolipids in presence of water-ethyleneglycol mixture. Temperature ranges of both peaks corresponded to ones on thermograms of dry glycolipids. Peak maximum temperature of sharp peak, observed both under cooling and heating of hydrated glycolipids, was the highest for monogalactosyldyacylglycerol and the lowest for sulfoquinovosyldiacylglycerol depending previously from its sugar groups and also from fatty acid composition. Interesting, that the contrary dependence between peak maximum temperatures and polar groups of glycolipids was observed for dry samples.

13-7

Studies on the alteration of native membrane and lipid bilayer properties by thioridazine.

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Thioridazine (TDZ) is a phenothiazine derivative clinically used as an antipsychotic drug. It is also known to act as effective antibacterial and antiplasmid agent, inhibitor of cancer cell growth, multidrug resistance modulator and putative antiprion drug. Our studies were performed to get some insight into possible molecular mechanisms of TDZ actions on the level of cell membrane. According to our molecular modelling calculations and experimental results (partition coefficient investigation) TDZ is less lipophilic than trifluoperazine or chlorpromazine. It interacts, however, with lipid bilayers, what was proved by microcalorimetric and fluorescence spectroscopic measurements. Phase transition properties of phosphatidylglycerol (bearing negative charge) were changed by TDZ to a greater extend than those of phosphatidylcholine (zwitterionic). Fluorescence polarization of DPH (inserted into liposome membranes) was increased in the presence of TDZ in concentration dependent manner when lipids were in liquid-crystalline phase, while almost no effects were observed when membranes were in the gel phase. Using Laurdan fluorescence we have also characterised the influence of TDZ on the properties of phosphatidylcholine liposomes containing different amounts of cholesterol. Like for DPH also Laurdan fluorescence anisotropy and generalised polarization were altered by TDZ for membranes in liquid-crystalline phase while almost no changes were observed for gel phase. Studies on Laurdan fluorescence anisotropy and generalised polarization dependence on temperature showed that membrane fluidity is perturbed by TDZ molecules in different way than phospholipid order. Using BCPCF efflux assay we surprisingly found that TDZ (unlike other phenothiazine derivatives) enhances dye efflux i.e. stimulates MRP1 protein in erythrocytes.

13-8

Mathematical model describing the thermotropic main phase transition in phospholipid membranes

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The fluidity of the lipid constituents is one of the most important factor associated with morphological changes in the biological membranes. Dipalmitoylphosphatidylcholine (DPPC) dimiristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylglycerol (DPPG) are used in this study as model-membranes in trials to evidence perturbative actions of temperature. Experimental data were obtained in a large interval of temperatures [25-70]0C. The model membranes pass from the gel phase to a liquid crystalline phase with temperature increasing. A pretransition and a main phase transition were evidenced. At the main phase transition temperature, the number of systems in the gel phase equalises the number of the systems in the liquid crystalline phase. The phase transition is reversible, showing a hysteresis loop dependent on the phospholipid structure. In order to estimate temperature dependence of the number of the systems from the gel and liquid crystalline phases, a mathematical model has been developed. In this model the average statistical weights of the gel and liquid crystalline systems are expressed as a function of spectral shifts measured in FTIR spectraof the model membranes. The IR signals corresponding to the symmetric and asymmetric CH2 stretching vibration modes of the acyl chains were used as indicators of the order degree in the model-membranes. The mathematical functions permit to establish with a very good precision the coordinates of the main phase transition (in the plan wavenumber and temperature). Acknowledgements Partial financial support of this study by TUBITAK Committee of Turkey in a NATO PC-B Programme is gratefully acknowledged by prof. dr. Dana Dorohoi

Posters

- Membrane structure and dynamics -

13-9

A peptide model of the mechanosensitive channel mscl investigated by molecular dynamics simulations and solid state nuclear magnetic resonance

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The mechanosensitive channel MscL plays an important role in the response of bacterial cells to osmotic shock. In its inactive state, the pore of this pentameric channel protein is held closed by a hydrophobic lock. However, a large pore is formed to release pressure in the event of osmotic shock. These large structural changes are induced by a change in membrane tension and hydrophobic thickness, thus lipid-protein interactions play a vital role for the function of MscL. To gain insight into the role of the hydrophobic lock and the influence of the membrane environment on the function of the protein, we studied a peptide model consisting of the pore lining alpha-helix TM1. Molecular dynamics simulations of the TM1 segment and derivatives with mutations in the region of the hydrophobic lock were conducted to evaluate this peptide model. To study the propensity of the petide to form bundles similar to the entire protein, pentameric helix assemblies were modelled using the crystal structure of the closed MscL protein as a template. The evolution of four peptide bundles, each formed by a different mutation in the interface region, was monitored during a 5ns molecular dynamics simulation to assess the stability of the bundle. In parallel, the influence of the peptide on the lipid environment was studied by deuterium solid state NMR. To this aim, the 30 amino acid sequence was synthesized by solid phase synthesis and reconstituted in chain-deuterated DMPC bilayers at different lipid:peptide ratios. First results show a significant change in the deuterium NMR spectra already at lipid:peptide ratios of 200:1, indicating successfull reconstitution of the peptide and a strong interaction of the peptide with the lipid chains.

13-10

Shape bistability can account for flickering of a membrane neck conductance

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Membrane topological transformations during endo- and exocytosis converge to a common intermediate - a thin membrane neck connecting a vesicle and plasma membrane. Ionic permeability of such necks (termed fusion or fission pores) often fluctuates between quasi-stable states. Fast jumps of the neck conductance or flicker were detected in secretory and nerve cells, as well as during virus-induced fusion. We detected flickering of both fusion and fission pore conductances in cultured macrophages IC-21. The conductance repetitively switched between two distinct levels. We found that such a behavior of the neck conductance can be reproduced in a simple system where the neck is modeled by a lipid membrane tubule extended between two rigid axisymmetric rings. Within a range of lengths the tubule has two stable shapes, catenoidal microtubule and cylindrical nanotubule. Their conductances can differ by up to four orders of magnitude. The transitions between the shapes occur spontaneously, resulting in conductance flicker, or can be controlled parametrically. The latter suggests that the tubule connection can be operated as a conductivity microswitch toggling the release of vesicle content in such cellular processes as kiss-and-run exocytosis.

13-11

Thermotropic phase transition in systems of dipalmitoylphosphatidyl-glycerol with gramicidin s

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We present a computational model for the experimental data recorded by Fourier Transform Infrared Spectroscopy regarding the influence of the antimicrobial peptide gramicidin (GS) concentration on the thermotropic phase transition of dipalmitoylphosphatidyl-glycerol (DPPG) lipid bilayer membrane in DPPG/GS systems. The model is based on the influence of the GS concentration on parameters of a nonlinear damped oscillator modeling the CH2 symmetric stretching band. One of the major assumption of our model admits that in the highly organized gel phase the influence of the GS presence is mainly on the damping coefficient and less on the potential distribution, while in the less organized liquid crystal phase, besides influence on the damping coefficient, an important change in the potential distribution is induced. The samples of DPPG/GS systems obtained from a stock solution of GS in ethanol were investigated in a large temperature range with increasing and decreasing temperature between 26.5 deg.C and 61.1 deg.C. The main observed effects of the gramicidin S presence are: a gradual decreasing in the magnitude of the bandwidth jump with increasing concentration, a translation of the whole hysteresis loop towards higher values of the bandwidth and an asymmetry of the decreasing temperature branch of the hysteresis loop. The results of the model are in satisfactory qualitative agreement with our experimental data.

13-12

High resolution proton MAOSS solid-state NMR: advantages on structural determination in biomembrane proteins

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Strong dipolar 1H-1H couplings cause difficulties in structural determination of biomembrane peptides or proteins. Until now, different methodologies and techniques have been developed to either increase the sensitivity or to produce highly resolved spectra using 1H as an indirect dimension. Recently, Magic Angle-Oriented Sample Spinning (MAOSS) has been demonstrated to enhance the resolution of 13C, 15N and 1H spectra, with especially effective line-narrowing observed for the latter through an averaging of the strong dipolar 1H-1H couplings and orientational imperfections (1). 1H NOESY, acquired under MAOSS at 400 Hz, has enabled the full assignment of an oriented DMPC sample. This new approach has been extended to three membrane active peptides: gramicidin (15 residues), melittin (26 residues) and beta-amyloid (39 residues). Cross peaks within amide-amide or amide-alphatic regions have been observed, however, due to the severe overlap in the alphatic regions, making sequential assignment a challenge. Recently, a four residue peptide was synthesised and incorporated into lipid membrane environment and this has been studied using 2D 1H MAOSS. Comparison between spectra acquired by solution NMR and MAOSS solid state NMR are made. The results indicated that structural studies are possible for a short peptide using this methodology. The high 1H resolution achieved by MAOSS has been found to be advantageous in multi-dimensional correlation experiment involving protons. (1) Glaubitz and Watts, J. Mag. Reson. 1998, 130: 305-316

Posters

- Membrane structure and dynamics -

13-13

Complete assignment of cholesterol in membranes By 2D MAS solid state NMR ; interpretation of 1h and 13C chemical shifts variations in terms of hydrog

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As typically performed in solution state NMR, complete assignment of proton and carbon resonances is an important issue in solid state NMR of membrane components. We will present results obtained with DMPC-cholesterol and DMPC-ergosterol (natural abundance, 10% and 100% 13C-labelled) from various MAS experiments at spinning rate ranging from 8 to 15 kHz: INEPT based 2D 1H-13C heteronuclear correlation(1), CP based HETCOR, INADEQUATE(2). Combination of these experiments has allowed for complete assignment of both sterols in their membrane environment. The comparison of carbon chemical shifts in organic solvent and in membranes has revealed specific chemical shifts variations, mainly localised in the first two ring of the sterol molecules. They were analysed in terms of hydrogen bonding and rotameric states of the hydroxyl group, using ab initio calculations of the isotropic chemical shifts. Similarly, using quantum mechanical calculations of static chemical shift anisotropy tensors, and comparing these with the experimental motionally averaged CSAs, we could extract orientational constraints sufficient to characterise completely the sterol's dynamics (average orientation, molecular order parameter). (1)Soubias et al., J. Magn. Res., 158, 143-148 (2002) (2)Lesage et al., JACS, 121, 10987-10993 (1999)

13-14

Spontaneous fusion of lipid vesicles with electropermeabilised cells

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Spontaneous fusion is observed between electropermeabilised cells and lipid vesicles. This is experimentally detected by the transfer of a soluble fluorescent dye, pyranin, trapped in the vesicles to the cell cytoplasm (content mixing). A homogeneous cytoplasm labelling is observed under digitised videomicroscopy. PS containing liposomes are brought in contact with the cells by a Ca2+ mediated electrostatic interaction. Pulsing conditions (electrical parameters, buffer) preserve cell viability. We demonstrated previously that spontaneous fusion occurred between electropermeabilised cells (1). But under the present conditions, liposomes are too small to be affected by the field (2). Fusion in the present study is obtained between an electropermeabilised cell surface and a metastable bilayer (LUV, SUV). Fusion is obtained not only by pulsing a mixture of cells and liposomes but simply by adding liposomes to prepulsed cells. This observation appears as a first experimental evidence of the hypothesis of Rosenheck that the first step triggering fusion in exocytosis, when vesicles are docked to the plasma membrane, was an electrostatic destabilisation of one partner (3). This work was supported by the PCV CNRS, ARC and Procope programs 1- Teissie and Ramos (1998) Biophys. J. 74:1889 2- Teissie and Tsong (1981) Biochemistry 20:1548 3- Rosenheck (1998) Biophys. J. 75:1237

13-15

Lateral Membrane Tensions Reduce Membrane Voltage Threshold Required For Electropermeabilization Triggering.

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Very little is known about the molecular mechanisms supporting living cell membrane transient permeabilization induced by fast trans-membrane electric field increase, as obtained by cell exposure to brief and intense external electric field pulses (electropermeabilisation). An electro-induced membrane electric potential difference is created that is position dependent on the cell surface. Its magnitude is controlled by the field strength, the cell radius and the dielectric properties of the plasma membrane. When the new membrane electric potential difference locally reached a critical value, a local alteration of the membrane structure leads to reversible permeabilisation. We attempted to determine whether mechanical tension could modulate membrane electropermeabilization. In our study, change in lateral tension of Chinese Hamster Ovary cell membrane has been osmotically induced. Suspending cells in hypoosmotic medium triggered transient cell swelling and associated membrane-cell surface increase (DS = 14%). Cell electropermeabilization was performed in the minute time range after the osmotic stress, i.e. before the regulatory volume decrease being activated by the cell. Time courses of living cell electropermeabilization were analyzed on cell populations using flow cytometry, and at the single cell level using a fast and sensitive fluorescent imaging acquisition system. We observed that electropermeabilization triggering was significantly facilitated when the lateral membrane tension was increased. The main conclusion was that the critical value of membrane potential required to trigger membrane electropermeabilization, was smaller when the membrane was under mechanical lateral constraint. This supports the hypothesis that both mechanical and electrical constraints play a synergetic role in transient local membrane destabilization.

13-16

Domain Formation In Model Lipid "Raft" Mixtures: Role Of Lipid Unsaturation

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There has been a great deal of interest in the interaction of cholesterol with lipids and lipid mixtures due to the importance of cholesterol in the formation of lipid domains or "rafts". It is generally believed that one requirement for raft formation in phospholipid-sphingolipid-cholesterol mixtures is the unsaturation of the phospholipid or non-raft forming component. However, there is conflicting evidence regarding the possibility for domain formation in mixtures containing lipids with a single monounsaturated chain; results of Dietrich, et al. (2002, Biophys. J., 80: 1417) on lipid monolayers suggest that mixtures containing monounsaturated lipids can form raft-like domains, while calorimetric studies by Shaikh, et al. (2002, Biochemistry, 41: 10593) suggest the opposite. Here, we investigate properties of model rafts, using mixtures of either SOPC or DOPC with cholesterol and egg sphingomyelin or DPPC, whose acyl chain composition is similar to egg sphingomyelin. From calorimetric measurements, we are able to determine those proportions of lipid, for each type of mixture, that are capable of forming lipid rafts, or coexisting liquid-ordered and liquid-disordered phases. Preliminary results demonstrate that DOPC is much more effective in forming rafts than SOPC.

Posters

- Membrane Structure and Dynamics -

13-17

Mechanical force required for membrane protrusion formation

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Cell morphology is determined mechanically by cytoskeletal networks of microtubules (MTs) and actin filaments. To study the mechanism of morphogenesis of cells, we previously made a model liposome encapsulated tubulin. Transformation process of liposomes was visualized using dark-field light microscopy. When tubulin was polymerized into MTs, spherical liposomes transformed into rugby-ball shape due to the mechanical force generated by the MT polymerization. Then, tubular projections of membrane grew from both ends of the rugby-ball liposome, called bipolar shape. In this experimental system, however, we could not measure the mechanical force required for the membrane transformation. Therefore, to analyze the transformation process of membrane vesicles quantitatively, we have developed a method to change the liposome shape by controllable mechanical force. We also developed the method to prepare liposomes that encapsulated a few polystyrene beads. By using double beam laser tweezers, mechanical force was applied onto the beads as to push liposome membrane from inside. With increasing the applied force, a spherical liposome transformed into rugby-ball shape, then tubular projection of membrane grew from either end of the rugby-ball liposome. Just after a membrane projection was made, the rugby-ball portion returned to spherical in shape. During the elongation of the rugby-ball shape, the stronger force became required with increasing its end-to-end length. However, once a protrusion was generated, the mechanical force necessary for elongating the protrusion became small and constant. These results indicate that membrane protrusion formation is based on the unique characteristics of two-

13-18

Alterations In Red Blood Cell Membrane Of Patients With Artificial Heart Valve

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Permanent interactions between artificial heart valve and morphotic elements in human blood can lead to changes in the plasma membrane of blood cells including red blood cells. The blood flow through the artificial heart valve is determined by higher value of shear stresses acting on the cell surface and in consequence by changes in the structure of membrane lipids and proteins. Furthermore, changes in blood cells are associated with generation of reactive oxygen species that can also induce damage to erythrocytes. The aim of the study was to investigate changes in red blood cells plasma membrane properties of patients with an artificial heart valve using electron paramagnetic resonance spectroscopy. Spin labeling and spectroscopic methods to evaluate red blood cell membrane fluidity and membrane protein conformation have been used. Have been found using spin labeled fatty acids (5-, 12- and 16-doxylstearic acid) we have found a significant increase in the membrane fluidity (5-DS $p<0,002$; 12-DS $p<0,002$ vs. control) and the significant changes in membrane protein conformation (for labels: maleimide $p<0,002$ and iodoacetamide $p<0,002$ vs. control). These results suggest that mechanical heart valves effect on red blood cells plasma membrane properties. Another possibility of changes in plasma membrane can be effected of drugs in therapy of these patients. This problem will explain in next research. Changes in lipid membrane fluidity and membrane protein conformations induced by artificial heart valves may result in red blood cells deformation during the blood flow through artificial valves.

13-19

Effect of new flavonoids on properties of lipid membranes and their ability to influence multidrug transporter acivity

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Multidrug resistance (MDR) of cancer cells is caused by outward active transport of anticancer drugs across plasma membrane carried out by MDR transporters (e.g. P-gp and MRP1). Activity of MDR transporters is strongly dependent on their lipid environment. The aim of presented studies was to find effective inhibitors of MRP-like transporter (MRP1) among new, extracted from plants flawonoids (isoflavones and flavones) and compounds that were obtained by chemical modification of genistein. Carboxyfluorescein derivative (BCPCF), that was proved to be a substrate of multidrug transporter MRP1, was used in functional assay to study of MRP1 transport activity. Effective MRP1 inhibitors extracted from Sophora species usually contain hydrophobic part in their molecules, like for example prenylgenisteins. Also several new genistein derivatives that are much more hydrophobic than precursor compound were studied and some of them proved to be very effective inhibitors of MRP1 activity. Function of membrane multidrug transporters is strongly related to surrounding lipids. To recognize to what extent flavonoids influence lipid bilayers, microcalorimetry and fluorescence spectroscopy were applied. Many of studied flavonoids strongly disturb fluidity of lipid model membranes. The ability of these compounds to perturb lipid thermotropic behaviour is dependent on position of hydroxyl and methoxyl groups in isoflavone or flavone molecules and on their lipophilicity. Dynamic light scattering was applied in studies of liposome aggregation in the presence of those isoflavones which promote aggregation. Comparison was performed between the influence of flavonoids on biophysical properties of lipid bilayers and inhibitory activity of these compounds against MRP1 efflux pump.

13-20

Domains in Membranes: Effects of Farnesol

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Farnesol is a 15-carbon isoprenoid which has a wide range of biological effects. From inducing apoptosis to activating transcription factors, this central player in the mevalonate pathway also post-translationally modifies proteins. The attachment of farnesol to proteins is essential for membrane-protein association and protein targeting. Studies of lateral membrane-farnesol organization are thus of interest. Confocal microscopy is used to visualize DMPC and DPPC giant unilamellar vesicles (GUVs) containing varying concentrations of farnesol. The observed domain formation is interpreted in terms of the DMPC-farnesol phase diagram . Effects of farnesol on the size and shape of lipid domains in sphingolipid-cholesterol-phospholipid membranes is also determined.

Posters
- Membrane structure and dynamics -

13-21

Tethered Lipid Bilayer Systems: Structure And Funcionallity

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We will present an overview of our work on tethered lipid bilayer systems. We try to mimic biological membrane functionality, namely electrical properties and the incorporation of functional transmembrane proteins. Tethering of the membrane architecture to a flat gold surface is performed by use of thiolipids. Special attention is paid to obtain a gold surface of high quality in terms of flatness by using a template stripping procedure from silicon wafer as a template. The sub-membrane region is build by lipid spacer molecules or a polymeric cushion. The upper part of the bilayer is completed by vesicle fusion or Langmuir-Blodgett/Langmuir-Schäfer transfer. Processes described so far are followed by Surface Plasmon Resonance (SPR), fluorescence microscopy, fluorescence recovery after photo bleaching (FRAP) and Electrochemical Impedance Spectroscopy (EIS). Stable systems with satisfying electrical sealing properties could be obtained. The incorporation and activity of valinomycin, gramicidin A and Cytochrome C oxidase in the tethered membrane could be investigated.

13-22

Hemifusion of giant vesicles: characterization of an intermediate state towards fusion induced by specific forces

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We studied the interaction between two giant vesicles to model membrane fusion, a biological process involved in viral infection and in intra- and extra-cellular communication. Fusion in the cell is induced by the action of fusogenic proteins which bring closer two opposing membranes to a short intermembrane distance. In our system, the bringing together of the membranes is induced by a specific attraction between functionalized lipids carrying adenine or thymine nucleotides on their head. We numerically estimated the equilibrium distance between two membranes, which decreases from 2.6 nm without specific forces to 1.4 nm when specific forces are present. Two vesicles are micromanipulated in order to place them into contact. A partial mixing of the lipids and an independence of the inner mediums are observed by fluorescence microscopy. The partial mixing of the lipids is quantified; it corresponds to a complete mixing of the external monolayers with an independence of the internal monolayers. These observations are characteristic of an intermediate state towards fusion: hemifusion. The kinetics of redistribution of the lipids between the two vesicles is measured; it is independent of the proportion of functionalized lipids, but agrees well in most cases with a model of diffusion on a “peanut”. This agreement is consistent with the presence of at least a few stalks at the interface between the vesicles. Some full-fusion events are sometimes observed, which open up new prospects for obtaining reproducible full-fusion in our system.

13-23

Influence Of Biologically Active Compounds On Thermotropic Phase Behaviour And Structure Of Phospholipid Model Membranes

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Organometallic compounds (MC) and amphiphilic quaternary ammonium salts (QAS) show a wide variety of biological activities. Among others, they are bactericidal (QAS), fungicidal (QAS, MC) and antitumor (MC) agents. The toxicity may be in part due to their interaction with membranes and in consequence alteration of the structure of membranes. We found (1,2) that diphenyltin dichloride (DPhT) and triphenyltin chloride (TPT) and various QAS significantly affect phase transition and structure of phospholipid bilayers. DPhT induced interdigitated phase formation and TPhT an inverted hexagonal phase. Also, the newly synthesised N-alkyl-N-carbalkoxymethylpiperidinium chlorides (strong microbial agents) and triphenyltin and tributyltin complexes with 3,4-diaminobenzoate and 2-[4-(dimethyloamino)phenylazo]benzoate (very effective cytostatic agents) affect the phase transition of DPPC. Very interesting properties exhibit mixtures of MC and QAS. For example, dodecyltrimethylammonium chloride decreases the ability of phenyltin compounds to induce structural changes in the phospholipid bilayer (3). 1. Rozycka-Roszak B., Pruchnik H. and Kaminski E. (2000), Appl. Organometall. Chem. 14, 465-472 2. Rozycka-Roszak B. and Pruchnik H. (2001) Appl. Organometall. Chem., 15, 233-235 3. Rozycka-Roszak B. and Pruchnik H. (2001) Z. Naturforsch. 56 c, 623-628

13-24

Dynamical Properties Of Lipoplex-Dna Gene Vectors

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The study of structural and dynamical properties of lipoplex-DNA compounds is nowadays of high scientific interest due to its potential application in somatic gene therapy, where they may be used as non-viral gene delivery vectors. Being their transfection efficiency related to the quantity of DNA entering a cell, it is of fundamental relevance to investigate the relationship between the composition of synthetic cationic liposomes (CLs) and the concentration of DNA. For this purpose, we have studied the H-bonded dynamics in the highly oriented lamellar DOTAP/DOPC-DNA model membranes as a function of the cationic lipid/DNA ratio, by incoherent neutron scattering. This technique provides unique information on the dynamics of biological macromolecules, probing the thermal excitations and bond distances characteristic of those systems. Results on the effect of the addition of DNA on the anisotropy of membrane dynamics will be presented together with a direct comparison with results observed on dynamic relaxation processes in pure DOTAP/DOPE as a function of the DOPE concentrations obtained using anelastic spectroscopy. 1) C. Castellano, D. Pozzi, G. Caracciolo, R. Cantelli, Appl. Phys. Lett., in progr. 2) F. Natali, A. Gliozzi, R. Rolandi, A. Relini, P.Cavatorta, A.Deriu, P. Riccio, A.Fasano (2000) Physica B, 301, 145 3) G. Caracciolo, R. Caminiti, F. Natali, A. Congiu Castellano (2002) Chem. Phys. Lett., 366, 200 4) F. Natali, A. Gliozzi, R. Rolandi, A. Relini, P.Cavatorta, A.Deriu. P. Riccio, A. Fasano (2002) App. Phys. A Mat. Sci. & Proc., 74, s01, s1582

Posters

- Membrane structure and dynamics -

13-25

The effect induced by the myelin basic protein on the proton dynamics in model membranes

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In order to understand pathological myelin-sheath breakdown, which is responsible for the loss of impulse conduction in Central and Peripherical Nervous System, it is of outmost importance to achieve a better knowledge of the key role of the myelin basic protein (MBP, its second most abundant protein) in determining its assembly, stabilization and compaction. Our elastic and quasi-elastic neutron scattering experiments performed on model membranes, show that the addition of physiological amounts of MBP (5 per cent in weight) induces a change in the average proton membrane dynamics across the lipid gel to liquid-crystalline phase transition. In particular, the appearance of anysotropic motion has been observed at two different time scales (explorable with IN13 and IN16 high energy resolution backscattering spectrometers at the nuclear reactor Institut Laue Langevin in Grenoble). Very recently the investigation has been extended to the study of the effect on model membrane dynamics induced by the protein in lipid-bound form, namely extracted with its endogenous lipids, in a form probably more close at the physiological one. For this purpose, elastic experiments have been performed over a large temperature range. References Gliss C. et al., (1999) Biophys. J., 77, 331 Konig S. et al., (1992) J. Phys. II France, 2, 1589 Natali F et al., (2001) Physica B, 301, 145 Natali F. et al., (2002) Appl. Phys. A, 74, S1582 Natali F. et al., (2003), Submitted to Chem. Phys Riccio P. et al., (1986) Biochem.Biophys. Res. Commun., 134, 313

13-26

Influence of biologically active compounds on thermotropic phase behaviour and structure of phospholipid model membranes

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Organometallic compounds (MC) and amphiphilic quaternary ammonium salts (QAS) show a wide variety of biological activities. Among others, they are bactericidal (QAS), fungicidal (QAS, MC) and antitumor (MC) agents. The toxicity may be in part due to their interaction with membranes and in consequence alteration of the structure of membranes. We found (1,2) that diphenyltin dichloride (DPhT) and triphenyltin chloride (TPT) and various QAS significantly affect phase transition and structure of phospholipid bilayers. DPhT induced interdigitated phase formation and TPhT an inverted hexagonal phase. Also, the newly synthesised N-alkyl-N-carbalkoxymethylpiperidinium chlorides (strong microbial agents) and triphenyltin and tributyltin complexes with 3,4-diaminobenzoate and 2-[4-(dimethyloamino)phenylazo]benzoate (very effective cytostatic agents) affect the phase transition of DPPC. Very interesting properties exhibit mixtures of MC and QAS. For example, dodecyltrimethylammonium chloride decreases the ability of phenyltin compounds to induce structural changes in the phospholipid bilayer (3). 1. Rozycka-Roszak B., Pruchnik H. and Kaminski E. (2000), Appl. Organometall. Chem. 14, 465-472 2. Rozycka-Roszak B. and Pruchnik H. (2001) Appl. Organometall. Chem., 15, 233-235 3. Rozycka-Roszak B. and Pruchnik H. (2001) Z. Naturforsch. 56 c, 623-628

13-27

Interaction of noscomin with model membranes

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Cyanobacteria are known to be a rich source of secondary metabolites with a wide variety of biological activities, including toxins, antibiotics, fungicides and antineoplasic agents. However, the occurrence of terpenoids in cyanobacteria is rather uncommon. Recently, a novel extracellular metabolite with an unprecedented diterpenoid skeleton, noscomin, has been isolated from Nostoc commune Vaucher by means of bioguided selection. In this work we present our studies of the effect of noscomin on biomembrane model systems by using infrared and fluorescence spectroscopies as well as differential scanning calorimetry. We show that noscomin interacts with membrane vesicles composed of different phospholipid composition, affecting the biophysical properties of phospholipid model membranes. The changes that noscomin promotes in the physical properties of model membranes, compromising the functional integrity of the cell membrane, could explain its antibacterial effects.

13-28

Instability of inhomogeneous membranes

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The recent discovery of a lateral organization in cell membranes due to small structures called 'rafts' has motivated a lot of biological but also physico-chemical studies. A recent experiment on model system has shown a spectacular budding process with the expulsion of one or two rafts induced by the absorption of proteins. A physical interpretation of the budding of the raft phase will be given. An approach based on the energy of the system including the presence of proteins is used to derive a shape equation and to study possible instabilities. This model shows various situations, strongly dependent of the nature of the proteins. In particular, a regime of easy budding when the proteins are strongly coupled to the membrane and a regime of difficult budding. We guess that this model gives a physical evidence for protein transportation by raft in the cell.

Posters

- Membrane structure and dynamics -

13-29

Structural changes in bacteriorhodopsin caused by mutation of the extracellular prolines

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Bacteriorhodopsin (bR) from Halobacterium salinarum is a proton pumping protein, forming seven transmembrane alpha-helices and containing a retinal molecule covalently bound through a protonated Schiff base (SB) to a lysine residue. Activated by light, the bR is able to translocate protons from inside to the outside of the cell, creating an electrochemical gradient used by the ATPsythase to generate ATP. The extracellular region of bR contains the prolines 8, 77 and 200 that are forming a pocket filled by some water molecules. Mutation of the prolines 8 and 77 to glicine, or Pro8 to Trp, cause an altered structure of the protein, indicated by the increased accessibility of hydroxylamine to the SB, and the decreased thermal stability of the protein. For the P8W, not only these effects are more important, but also the retinal environment is strongly altered, as is shown by the appearance of the purple acid form in the absence of chloride and the early SB deprotonation. These structural changes are interpreted as an opening of the extracellular region and increased flexibility, allowing water molecules to enter near the retinal. This hypothesis is in agreement with the fact that almost all these mutants show an altered Asp 85 pKa, principal counter-ion of the SB. Therefore, these prolines are important in the maintenance of the correct helical packing, most likely through the preservation of the adequate hydrogen bonding network in the extracellular region of bR.

13-30

Small-angle x-ray and neutron scattering studies of thylakoid membrane structure and structural flexibility

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Chloroplast thylakoid membranes of higher plants form a highly organised stacked multilamellar system, which is differentiated into granum and stroma regions and highly enriched in membrane bound protein complexes constituting the photosynthetic machinery. Our study addresses the structure of the thylakoid membrane system on a lengthscale of the order of 10 nm and the question of structural reorganisations upon environmental changes, e.g. changes in osmolarity, ionic strength and illumination. Thylakoid membranes were freshly isolated from spinach or pea leaves and oriented in a magnetic field. Small-angle X-ray scattering reveals two peaks in the region 0.5 - 2 nm-1. The overall shape of these peaks is mainly determined by the grana formfactor which in turn depends on the electron density distribution in the flattened vesicles constituting the grana stacks. Modelling of the data shows that application of an osmotic pressure results in a decrease of the inter-thylakoid distance leaving the lumen thickness unaltered, while pre-illumination in strong light increases the inter-thylakoid distance. These results are confirmed by small-angle neutron scattering. Dark adapted thylakoid membranes suspended in a standard medium are characterised by a stroma repeat distance of 31.6 nm and a grana repeat distance of 18.3 nm. Extended pre-illumination in strong light increases the stroma repeat distance with 2.5 nm. In contrast, short illuminations with more moderate light intensity during the data collection are shown to decrease the stroma repeat distance by 1.4 nm. Dark adaptation of the thylakoid membranes after short illuminations shows that these structural changes can be reversed

13-31

3-dimensional organization of cubic membranes: study of endoplasmic reticulum paracrystals in photosomes

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It is an old challenge to understand the true 3-D structure of the photosomes which are the sites of bioluminescence in an annelid, Harmothoe lunulata. These photosomes are arrangements of coiled endoplasmic reticulum tubules (ER) forming para-crystals, which are permanent but evolutive structures. The ER membrane bears a photoprotein which emits light when triggered by oxygen radicals. The convoluted membranes of ER are particularly well preserved by the ultrarapid freeze-fixation (slam-freezing) which facilitates their image analysis. The micrographs of thin sections of the photosomes show regularly organized domains that correspond to randomly oriented slices of a 3-D crystal with a cubic symmetry of aspect Q4 (Pn..). The lattice parameter (about 50 nm) of this "cubic ER membrane(s) system" was deduced from freeze-fracture images. This cubic organisation of the ER membranes has been found in many others subcellular organelles and combines several general advantages: It separates the subcellular space into two unconnected parts (labyrinths); The membrane surface between those laryrinths is maximised while the stabilisation energy is minimised. Our results also suggest that some kind of ER cubic polymorphism may be observed during system activation.

13-32

Study of the interaction of biologically active dicatechols deriving from an african potatoe extract (hypoxis rooperi) with phospholipid model membran

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The rhizome of the plant Hypoxis rooperi has been studied due to its anti-inflammatory and anticarcinogenic activities. In the present study we have characterized an extract from H. rooperi by HPLC, isolating the dicatechol glucoside hypoxoside and obtaining its aglycone analog, rooperol, through enzymatic digestion. The effect of both compounds was studied in model membranes in order to contribute to explain its biological activity. Rooperol showed a much higher phospholipid/water partition (3.40 x104) than hypoxoside (0.11x104) suggesting a much more efficient incorporation into model membranes. The location of these dicatecols into PC bilayers was investigated through quenching fluorescence assays using lipophilic spin probes or acrylamide, and fluorescence resonance energy transfer (FRET) to PA-DPH experiments. These concluded that rooperol was more deeply located into the phospholipid membrane than hypoxoside, which was positioned nearest to the phospholipid/water interface, since rooperol showed a higher efficacy in FRET to the probe PA-DPH. Both molecules presented comparable Forster transfer radium (Ro). Differential scanning calorimetry studies (DSC) evidenced that rooperol showed a higher capacity than hyposoxide to disturb PC membrane structure by increasing the lipidic order in the fluid state and lowering enthalpy and cooperativity of its main phase transition. Rooperol effects on PE model membranes were also more pronounced consisting in an important decrease of the liquid-crystalline to hexagonal transition, fact that was corroborated by 31P-NMR studies. The present work postulate that some of biological activities described for rooperol could be attributed to the drastic changes

Posters

- Membrane structure and dynamics -

13-33

Sarcolemma phospholipid structure in normal and dystrophin deficient muscles studied by 1H and 31P NMR spectroscopy with lanthanide ions

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In Duchenne Muscular Dystrophy as in mdx mice and grmd dog muscles, genetically deficient in dystrophin, profound dysfunction and disruption affect plasma membrane (sarcolemma). Many studies have been carried out on protein alterations, but few is know about sarcolemma phospholipid structure. In particular, dystrophin is known to interact with phospholipids but we have no information about sarcolemma phospholipid structure in dystrophin deficient muscle. Normal membranes are known to exhibit an asymmetrical trans-bilayer distribution of lipids. This organisation plays a crucial role for membrane properties and function and lost of asymmetry is involved in cellular death and apoptosis. We investigated phospholipid structure of sarcolemma vesicles isolated from normal and dystrophin deficient muscles. The trans-bilayer phospholipid distribution was studied by static and magic angle spinning NMR spectroscopy. The use of lanthanide ions allowed to distinguish internal from external phospholipids. We demonstrated that sarcolemma phospholipid composition is not modified between normal and mdx mice, but is modified in grmd dog compared to normal. Phosphatidylcholine represent 65 percent of total phospholipids for normal dog, and only 45 percent for grmd dog. Sarcolemma vesicles sidedness is controlled with biochemical assay. Lanthanide ion addition induces a shift of NMR signal corresponding to phospholipids located on the outer side of the membranes while unshifted signal represents internal phospholipids. Knowing internal layer lipid content and assuming they represented fifty percent of total lipids, we are able to obtain sarcolemma external layer composition

13-34

Measuring membrane bending rigidity by micropipette aspiration

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The mechanical properties and behavior of lipid membranes have been studied intensively over the last decades both theoretically and experimentally. This has lead to the development of a variety of experimental methods and theoretical descriptions to characterize the complex response of these soft systems to external forces. However, discrepancies between results, obtained by different techniques, persist. In the present work the membrane bending rigidity is discussed in relation to results obtained by flicker-noise spectroscopy and micropipette aspiration techniques. A statistical mechanical model for weakly aspirated vesicles is presented, and analyzed for effects of system geometry, membrane pipette interactions, membrane asymmetry, choice of initial state, and membrane area stretch. The model and the predictions, obtained from numerical simulations of the aspiration process, are furthermore used in the interpretation of data from micropipette aspiration of SOPC vesicles.

13-35

Influence of helix-kinking residues of bacteriorhodopsin on the correlation between m intermediate formation and photocurrent components

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Bacteriorhodopsin (BR) is a proton pump consisting of 7 transmembrane alpha-helices embedded in the purple membrane of *Halobacterium salinarum*. A retinal molecule linked to the protein absorbs light, initiating a photocycle that leads to the pumping of a proton from the interior to the exterior of the cell. In the middle of helix C, there is a kink induced most likely by both Thr90 and Pro91, with a possible contribution of Asp115. In helix F, there is another kink induced solely by Pro186. In this work we present data on charge movements during the transition from L to M in the mutants T90A, D115A, P91A and P186A. By comparing flash photolysis and photocurrent data of oriented BR gels, the optical signals of the L-M transition were correlated to the kinetics of proton transfer from the Schiff Base (SB) to Asp85 and the concomitant release of the proton from the proton release group (PRG). All the mutants show faster charge movements than WT in the B2 phase, in agreement with faster M rise. However, D115A in the pH range 6.8-8.3 shows slower charge movements as compared to M rise, arguing for a loosening of the relationship between the optical intermediates and charge movements in this mutant. T90A displays a very fast and almost pH independent L-M transition and B2 photocurrent. The data highlight the importance of these residues in the maintenance of the native conformation of bacteriorhodopsin and their participation in light-induced proton translocation.

13-36

Effect of Sodium Chloride on a Lipid Bilayer

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Electrostatic interactions govern structural and dynamical properties of membranes and can vary considerably with the composition of the aqueous buffer. We studied the influence of sodium chloride on a pure POPC lipid bilayer by fluorescence correlation spectroscopy experiments and molecular dynamics simulations. Increasing sodium chloride concentration was found to decrease the self-diffusion of POPC lipids within the bilayer. Self-diffusion coefficients calculated from the 100 nanosecond simulations agree with those measured on a millisecond time scale, suggesting that most of the relaxation processes relevant for lipid diffusion are faster than the simulation time scale. As the dominant effect, the molecular dynamics simulations revealed a tight binding of sodium ions to the carbonyl oxygens of in average three lipids leading to larger complexes with reduced mobility. Additionally, the bilayer thickens by approx. 2A which increases the order parameter of the fatty acyl chains. Sodium binding alters the electrostatic potential which is largely compensated by a changed polarization of the aqueous medium and a lipid dipole reorientation.

Posters

- Membrane structure and dynamics -

13-37

Spontaneous Water Permeation Through a Lipid Bilayer

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Passive transport of molecules across biological membranes plays a vital role in cells. Whereas the unassisted passage of charged molecules is almost impossible, the transport of water molecules is essential for osmotic balance. Due to the low permeation rate, simulation studies of water permeation had up to now to rely on the validity of standard rate theory, requiring the usual assumptions. Here, we study for the first time the unperturbed and spontaneous passage of individual water molecules across an uncharged lipid bilayer by 'real time' molecular dynamics simulations. Several full permeation events have been observed and allowed the accurate first principles determination of rates and the associated free energy profile for the transport of water molecules across the membrane. In contrast to membrane channels, the motion is non-cooperative. The calculated diffusive water permeability coefficient ranges from 4 to 11*10^-4cm/s and is thus in excellent agreement with experimental results. The knowledge of both, the height of the activation barrier and the rate, allows for the calculation of the Kramer's pre-factor to 2-6 m^-2 s^-1.

13-38

Solid state nmr spectroscopy studies of the structure and dynamics of the liquid ordered phase

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Solid state NMR spectroscopy has been used to study structural properties of model lipid rafts in the liquid ordered phase for a series of equimolar phosphatidylcholine cholesterol and sphingomyelin cholesterol. Magic angle spinning was employed to aid resolution of 1H, 13C and 31P nuclei and temperature varied between 5oC and 60oC. Although most of these systems are thought to exist as a single phase at the temperatures studied, the liquid ordered phase showed a range of properties. Changes in temperature cause an increase in the conformational freedom of the glycerol region, lipid spacing and chain disorder. Splitting of the carbonyl group suggests hydrogen bonding from the cholesterol, while each of the double bond peaks of cholesterol are split into two indicating different environments. Analysis of the lipid chains in the 13C spectra suggest that two mechanisms operate as the temperature is raised; the first increases lipid spacing without effecting chain packing while the second disorders the acyl chains

13-39

Molecular dynamics simulations of the E1/E2 transmembrane spanning domain of the semliki forest virus

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Transmembrane helix-helix interactions are important for viral membrane fusion and budding. Here is studied the helical packing between the transmembrane spanning domains of the two glycoproteins E1 and E2 of the Semliki Forest virus. Molecular dynamics simulations were performed in the wild type E1/E2 complexes and in different mutations. The simulations revealed that the isolated wild type E1 peptide formed a more flexible helix than the rest of peptides and that the wild type E1/E2 complex consists of two helices that intimately pack their N-terminals. The residues at the interhelical interface showed a periodicity resembling that of left-handed coiled coils. They were small and medium residues as Gly, Ala, Ser and Leu, which also had the possibility to form interhelical Calpha-H777O hydrogen bonds. Results from the other complexes suggested that "correct" packing is a compromise between these residues at both E1 and E2 interhelical interfaces. This compromise can explain the low sequence identity at the transmembrane domain of the E2 peptide of the Semliki Forest virus and its related enveloped alphaviruses and it allowed the prediction of the putative contact residues at the transmembrane spanning domain of alphaviruses as Sindbis, Ross River, and Western and Venezuelan Equine Encephalitis virus.

13-40

Binary phase diagram of mixed chain phosphatidylethanolamine and phosphatidylglycerol.

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Phosphatidylethanolamines (PE) and phosphatidylglycerols (PG) are the most abundant phospholipids present in bacterial cytoplasmic membranes. Therefore, mixtures of PE/PG can be used as simple membrane mimetics for certain bacteria. Biophysical characterization of such models is relevant to understand the behaviour of membranes themselves, as well as their interaction with other molecules, for instance antimicrobial peptides.
A partial phase diagram of fully hydrated mixtures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (Na-salt) (POPG) in physiological buffer was established on the basis of the on- and offset temperatures of the phase transition, determined from the excess heat capacity curves. The main transition of lipid mixtures rich on POPE exhibited a high cooperativity, while the transition of mixtures with higher POPG content become broader, indicating loss of cooperativity. X-ray diffraction of pure POPE and POPG membranes showed the expected patterns for multilamellar (MLV) and unilamellar vesicles (ULV), respectively. However, POPE/POPG mixtures showed Bragg peaks of large lamellar repeat distance in the gel phase (L beta), whereas the typical pattern of uncorrelated bilayers was observed in the fluid phase (L alpha). We could relate this phenomenon to a discontinuous unbinding of lipid multibilayers, which appears to be driven by steric repulsion due to bilayer fluctuations [1], which are very pronounced in the L alpha phase.
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Posters

- Membrane structure and dynamics -

13-41

Using solid-state NMR to probe the order of unsaturated lipids in membranes, with and without cholesterol

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DROSS (Dipolar Recoupling On axis with Scaling and Shape preservation) is a solid-state NMR technique optimized for measuring ^{13}C-^{1}H dipolar couplings and order parameters in lipid membranes in the fluid phase (1). It was showed to be efficient in 1,2-dimirystoyl-sn-glycero-3-phosphocholine hydrated membranes. Here we show that it can also be applied to multilamellar vesicles containing unsaturated lipids, which are difficult to deuterate, or lipid mixtures, including cholesterol.
With such an approach we have been able to observe subtle phase changes by following the behavior of resonances around the double-bonds. Thereby, we have completed the picture of the *lo* phase obtained by ^{2}H NMR for DPPC (2) to POPC and DOPC which behave quite differently.
Because these observations are made with natural lipids and no external probe or isotopic labeling, our approach is compatible with observations of a *natural* biomembrane. In conclusion, we think that DROSS can be a useful tool to probe the existence of microdomains or rafts in biological membranes (3).

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13-42

3D structure and interactions of the neuropeptide met-enkephaline with negatively charged bicelle membranes

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The interaction of the neuropeptide methionine-enkephalin (Menk) with bicelles membranes (Bic) doped with zwitterionic (PE), or negatively charged (PS, PG) lipids was followed by solid sate NMR. Wide line ^{31}P and ^{2}H NMR demonstrated that Menk disorders selectively chains and head groups. Results suggest that the effect depends upon the insertion depth into membranes, which is modulated by a balance between hydrophobic and electrostatic interactions (*1*). ^{1}H Magic Angle Spinning NMR of the Bic/PS sytem afforded determination of the peptide 3D structure bound to the membrane. The use of this technique on perdeuterated bicelle membranes affords a resolution for peptide resonances akin to solution NMR (*2*). By adapting the battery of NMR sequences utilized for solution structure determination to MAS NMR we found that Menk undergoes a conformational change on going from the solution to the membrane. The through-space correlations found by NMR between lipids and Menk were injected into molecular mechanics calculations and led to a precise structure and localization of the neuropeptide in membranes.

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13-43

Sphingomyelinase activity in sphingomyelin cholesterol mixtures

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Sphingomyelinase activity was assayed on large unilamellar vesicles composed of sphingomyelin (sm)/ cholesterol (ch) mixtures at varyingproportions. natural (egg) sm was used with a gel-fluid transition temperature at ca. 40º c. when the enzyme was assayed at 37º c, the activity on pure sm was exceedingly low, but a small increase was observed as soon as some ch was added, and a large enhancement of activity occurred with Ch proportions above 25 mol %. The data were interpreted in terms of sphingomyelinase activity being higher in the cholesterol-induced liquid ordered phase than in the gel phase. The abrupt increase in activity above 25 mol % Ch would occur as a result of a change in domain connectivity, when the Ch-rich liquid ordered domains coalesced. In equimolar SM/Ch mixtures, that were in the liquid ordered state in a wide range of temperatures, sphingomyelinase activity was virtually constant in the 30-70º C range. The results demonstrate that at the mammalian and bird physiological temperatures Ch regulates sphingomyelinase activity, and that this can occur precisely because most SM have a gel-fluid transition temperature above the physiological temperature range. In addition, Ch activation of sphingomyelinase and the strong affinity of Ch for SM allow the rapid, localized and self-contained production of the metabolic signal ceramide

13-44

Behavior of GM3 ganglioside in lipid monolayers mimicking the raft composition

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Some lipids such as cholesterol and sphingolipids are able to form condensed domains called rafts in cellular membranes. In this work we study the interaction of the GM3 ganglioside with different lipids: sphingomyelin (SM), palmitoyl-oleoyl-phosphatidylcholine (POPC) or cholesterol, in order to better understand the interactions likely to exist in rafts.
Surface pressure measurements were performed on binary monolayers containing increasing amounts of GM3 and mean molecular areas were analysed. Their variation recorded on SM-GM3 and POPC-GM3 isotherms versus GM3 content were not drastically different: at high percentage (40 mol%) of GM3, a condensation of the monolayer is observed in both cases. At lower percentage (≤20 mol%), SM-GM3 and POPC-GM3 behave on the whole as "ideal" mixtures. This result is surprising since a stronger condensation effect was rather expected with SM-GM3 mixtures. Indeed SM is able to act as donnor and acceptor for hydrogen bonding and could build a hydrogen bonding network with GM3 (such a network is supposed to be important for the cohesion of molecules in rafts). POPC is only an acceptor and the network is less likely. These results could be explained by a different distribution of GM3, according to its amount, in the monolayers. Therefore fluorescence microscopy experiments are on the way to underline possible domain formation.
At last, first results obtained with cholesterol-GM3 or POPC monolayers show that cholesterol would be able to condense GM3. Experiments of cholesterol desorption by cyclodextrin

Posters
- Membrane Structure and Dynamics -

13-45

DHPC-DMPC-water phase diagram at high water content

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We have examined the dihexanoyl-phosphatidyl choline (DHPC) dimiristoyl-phosphatidyl choline (DMPC) water phase diagram at high water content to better understand the thermodynamics of this ternary system and the different amphiphile structures formed. This particular lipid detergent-water system is of particular interest as in addition to the normally observed mixed micelle and mixed bilayer phases it is capable of forming isotropic and anisotropic bicelle phases at intermediate water content as observed by nmr spectroscopy. The object of our study is to extend the observations that have been made at intermediate water content to high water content and better describe the phases boundaries in this system. Our analysis is based on the use of several complementary techniques to define the phase boundaries and examine the structures of the different phases identified. These techniques include titration and scanning calorimetry, static and dynamic light scattering, viscometry, infrared spectroscopy and electron microscopy. This collection of techniques gives a coherent view of the phase diagram including up to four different phases separated by three phase transitions. We hope that the knowledge of the phase structure of this system will facilitate the use of bicelles in solutions with high water content.

13-46

Interaction of surfactant protein A with KL4-Surfactant

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Pulmonary surfactant, a lipid-protein complex that covers the alveolar surface, prevents alveolar collapse and contributes to lung defense. The alteration or deficiency of this system leads to respiratory distress. Treatment with exogenous surfactants is useful to improve lung function. KL_4-Surfactant, is a novel synthetic "humanized" surfactant consisting of dipalmitoylphosphatidylcholine (DPPC), 1-palmitoyl-2-oleyl-phosphatidylglycerol (POPG), palmitic acid (PA) and a 21-residue synthetic amphipatic peptide, KL_4 that mimics human surfactant protein B.
The aim of this paper is to analyze the interaction of surfactant protein A with KL4-DPPC/POPG/PA liposomes. SP-A is implicated in multiple biological functions, including improvement of the biophysical activity of surfactant and host defense. Because recombinant human SP-A has the potential to be used as component of new surfactants, it is relevant to explore the interaction of this protein with KL_4-Surfactant.Temperature-dependent anisotropy measurements of 1,6-diphenyl-3,5-hexatriene (DPH) incorporated in DPPC/POPG/PA vesicles with or without KL4 indicated that KL4 increased the phase transition temperature of these vesicles. Addition of SP-A to DPPC/POPG/PA vesicles did not affect the thermotropic behavior of those vesicles. However, the interaction of SP-A with KL4-DPPC/POPG/PA vesicles reversed the increasing effect of KL4 on the transition temperature, possibly indicative of an interaction between the protein and the peptide. In addition, we found that KL4 induced Ca^{2+}-dependent aggregation of DPPC/POPG/PA vesicles at neutral and mildly acidic pH. Aggregation can be correlated with the rapid adsorption and spreading of KL4-DPPC/POPG/PA vesicles into an air-liquid interface in the presence of calcium. The presence of SP-A further increased the extent of KL4-surfactant aggregation.

13-47

Effects of levorin and mycoheptin on cation fluxes and conductance in frog muscle fibre membrane.

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The cation conductance and effluxes induced by fungicidal polyene antibiotics levorin (10^{-8}–$5\cdot10^{-5}$ mol/l) and mycoheptin ($1.3\cdot10^{-6}$–10^{-5} mol/l) on isolated frog skeletal muscle fibres and whole sartorius muscles of the frog have been investigated. Conductance was measured under current clamp conditions, using a double sucrose-gap technique. Cation effluxes were studied by means of flame emission photometry.
Both antibiotics increased the cation conductance and efflux rates, the effect of levorin being more pronounced. At a concentration of 10^{-5} mol/l mycoheptin- and levorin-induced conductance was $25.3\pm1.1\ \Omega^{-1}\cdot10^{-4}/cm^2$ and $42.3\pm2.1\ \Omega^{-1}\cdot10^{-4}/cm^2$, respectively. The potassium efflux rates induced by the polyenes at a concentration of $2.5\cdot10^{-5}$ mol/l were $2.49\pm0.11\cdot10^{-3}$ min^{-1} for mycoheptin and $5.19\pm0.34\cdot10^{-3}$ min^{-1} for levorin. The concentration dependence of mycoheptin-induced potassium conductance (1.3-fold) was shown to be lower than on artificial membranes, whereas aromatic heptaene levorin had an even smaller concentration dependence. The kinetics of release of the polyenes from isolated fibres and whole muscles was studied by rapid removal of the antibiotics from solution. The decline in the equilibrium conductance caused by mycoheptin removal was very fast (during the first minute $\tau = 2.39$ min). In contrast, levorin-induced conductance was irreversible. It is known that levorin and mycoheptin form ionic channels in lipid bilayers. Our results show that the processes which limit the rate of channel formation are different in biological and model membranes. It is suggested that levorin might cause toxic side effects resulting from the interaction with cell membranes.

13-48

Translocation of Phospholipids in K562 Cells expressing MDR1

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Spin-labeled lipids with a short β chain were introduced in the outer monolayer of suspended K562 cells (wt or adr+) and their translocation was determined by the back exchange technique[1]. In cells containing the P-glycoprotein (adr^+ cells), the rapid translocation from the outer to the inner leaflet of aminophospholipids by the aminophospholipid translocase was slowed down. PSC833, a specific inhibitor of the P-glycoprotein, reversed at least partly the effect. These experiments suggest that PS and PE are substrates of the P-glycoprotein although the outward transport by the MDR1 protein appears to be much less efficient than the inward transport by the aminophospholipid translocase. Sphingomyelin (SM) and phosphatidylcholine (PC) disappear from the outer surface at a slower rate by endocytosis[1]. For the latter lipids a difference of uptake between K562 (wt) and K562 (adr+) was barely detectable, at least during the first 30 min a 37°C. Further experiments with K562 cells were carried out to assess a possible role of the aminophospholipid translocase during the early stage of endocytosis[2]. To this end, we have carried out experiments employing simultaneously a ^{14}N spin-label and a ^{15}N spin-label[3]. One probe (PE or PS) was substrate of the aminophospholipid translocase and its concentration was varied, the second probe (PC or SM) was an indicator of endocytosis.

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Posters

- Membrane structure and dynamics -

13-49

Molecular dynamics investigations of preformed neu transmembrane dimers in a DMPC bilayer.

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Ligand-induced dimerization is a common property to the transmembrane signaling mechanism of receptor tyrosine kinase of the EGFR family. However, although dimerization is necessary for receptor activation a specific rotational orientation of the intracellular domains is crucial for tyrosine residue phosphorylation (1). The transmembrane domain is involved in dimer formation and plays a regulatory role in this flexible rotation process. The Neu receptor of the EGFR family is constitutively activated by the single amino acid change (Val664 to Glu) in the transmembrane domain which lead to cell transformation (2). Very likely the Glu residues attract each other through hydrogen bonding and induce a specific helix-helix packing defining rotational constraints for receptor activation.
In vacuo molecular dynamics simulations of the transmembrane domain of the receptor dimer reveal that the two helices pack with left handed interactions when the Glu residues are at the dimer interface (3, 4).
To better characterize the structure of the transmembrane domain we examine the behavior of several of these models embedded in a fully hydrated dimiristoylphosphatidylcholine (DMPC) bilayer model. Examination of the dynamic nature of these structures in DMPC environment, the characterization of helix-helix interactions and helix-lipid interactions may help to better understand the role of the Glu mutation in receptor dimerization and activation.

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13-50

Membranes grafted with long hydrophilic polymers

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We study the elastic properties of lipid membranes grafted with long hydrophilic polymers. Theoretical calculations predict two limiting regimes for the membrane spontaneous curvature as a function of the surface polymer concentration: i) at low coverage (mushroom regime) the spontaneous curvature scales linearly with the surface density of anchored polymers; ii) at high surface concentration (brush regime) the dependence is quadratic. In both regimes the bending stiffness of the membrane is expected to increase. We attempt to test these predictions by monitoring the morphological changes induced on giant vesicles.
Experimentally, we swell vesicles made of DOPC/biotinylated-DOPE lipids in presence of avidin. The solution media is then exchanged with a solution of fluorescently labeled λ-phage DNA molecules with biotinylated ends. The polymer (DNA) grafts to the membrane via a biotin-avidin-biotin linkage. By varying the amount of biotinylated DOPE we control the surface concentration of the anchors. The DNA grafting can be characterized with fluorescence measurements and isothermal titration calorimetry. Changes in the elastic properties of the membrane are observed as a function of the surface coverage. We estimate the spontaneous curvature and bending modulus of the membrane from analysis of the vesicle fluctuations. We observe an increase in the spontaneous curvature of the membrane as the surface concentration of DNA is increased. At higher grafting concentrations the vesicles bud. The size of the buds can be used to assess the membrane curvature. The effect on the bending stiffness is well pronounced and is a subject of further investigation.
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13-51

Adhesion and fusion of functionalized giant vesicles

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Adhesion and fusion of membranes has attracted interest from both biological and physical sciences, yet little is understood about the mechanism in which fusion actually occurs. We functionalize giant unilamellar vesicles with synthetic amphiphilic molecules bearing β-diketone and/or bipyridine ligands. These functional groups have a propensity to form complexes with multivalent ions (Eu^{3+}, Tb^{3+}, Ni^{2+}, Cu^{2+}) in 2 to 1 ligand to metal ratio. We study adhesion and fusion induced by formation of ligand-metal complex between two neighboring vesicles.
Using optical video microscopy and micropipette manipulation we attempt to (i) compare the effect of the multivalent metal ions on vesicles without the ligand molecules versus membranes with ligand molecules; and (ii) characterize the fusion process observed in functionalized vesicles in presence of ions. At low ion concentrations (< 1μM) we observe that the vesicles strongly adhere. At higher concentrations (~ 5μM) the vesicles rupture. We measure the contact angle between two adhered vesicles to estimate the energy associated with the membrane-ion interaction as a function of membrane tension. Functionalized vesicles are observed to fuse after locally injecting concentrated ionic solution. Fusion between two vesicles consistently takes place on the time scale of less than one second. In summary we demonstrate the influence of multivalent ions on the adhesion and fusion of giant vesicles funtionalized with hydrophilic β-diketone and bipyridine ligands.
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13-52

Membrane curvature and dynamics induced by insertion of oligomer amphiphiles

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We study the morphological changes in lipid membranes due to insertion of oligooxyethylene alkyl ethers, C_iE_j (e.g. $C_{10}E_{13}$, $C_{18}E_{100}$). The shapes and thermal fluctuations of giant vesicles are monitored as a function of bulk oligomer concentration via phase contrast microscopy. We exchange the solution media and observe the response of a selected vesicle. We employ a wide amphiphile concentration range below the respective CMCs.
Experimentally we quantify the surface concentration of C_iE_j by measuring the partition coefficient from bulk solution into the membrane employing Isothermal Titration Calorimetry (ITC) on small unilamellar vesicles. The insertion of $C_{13}E_{10}$ in the membrane of giant vesicles leads to an increase in the vesicle surface in correspondence to the partition coefficient measured with ITC. This increase indicates an exchange of material between the two monolayers composing the membrane. In the case of $C_{18}E_{100}$ vesicles have the tendency to bud presumably due to the larger hydrophilic headgroup of the oligomer. No increase in the vesicle surface area is detected.
Changes in membrane curvature and bending stiffness are quantified via analysis of vesicle fluctuations. Overall, the membrane softens with insertion of the amphiphiles. The membrane spontaneous curvature exhibits an interesting and complex dynamics. After initial insertion of $C_{13}E_{10}$ in the external monolayer of the membrane, we observe a drastic and quick curvature increase (~10 min) which then relaxes over a much longer time interval (few hours). We tentatively interpret these results as being due to establishing of adsorption/desorption equilibrium on both sides of the vesicle membrane.
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Posters
- Membrane structure and dynamics -

13-53

Model of effect of multivalent cations on neutral lipid membranes

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We analysed the effects of multivalent cations (MC) on the mechanical state of neutral lipid bilayers such as phosphatidylcholine (PC). Assuming that an adsorbed MC (AMC) forms a complex with the phosphate group (MC-PG) of the lipid head, we considered the following consequences of MC adsorption: 1) appearance of double electric layer (DEL) in solution; 2) local electrostatic interaction between MC-PG and the nearest neighboring lipid molecules; 3) change in surface hydration (determined from surface dipole potential). We have developed a model that describes the contributions of these effects to total chemical potential of a lipid membrane. The present findings indicate that DEL and electrostatic attraction between MC-PG and neighboring molecules provide the main contributions. DEL induces lateral expansion of the bilayer, but electrostatic attraction between MC-PG and neighboring molecules induces lateral compression of the bilayer. To compare our theoretical results with previously obtained experimental data, we considered the effect of La on the chain-melting phase transition temperature Tm of dipalmitoyl-PC (DPPC) membrane. From this comparison, we concluded the following: 1) bilayer local compression in the vicinity of the MC-PG is the cause of the increase in Tm; 2) the effect of attraction between MC-PG and nearby molecules is stronger than the effect of DEL, due to the low dielectric constant of the MC-PG environment. The effects of MC on the visicles formed by DPPC was considered in framework of the developed model.

13-54

Substrate-induced infrared difference spectra of melibiose permease from *E. coli*

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The melibiose permease (MelB) of *Escherichia coli* is one of the best known transporters of a large family of Na^+ solute symporters. MelB couples uphill transport of alpha and beta galactosides to the downhill inward movement of Na+, Li+ or H+. Experimental data strongly support a topological model of 12 transmembrane domains with the N and C termini located in the cytoplasm.
In this work we present a difference spectroscopy analysis of MelB conformational changes induced by substrate binding, by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). Buffers, containing MelB substrates (Na+/melibiosa and Na+/H+), were guided through a continuous flowing system over a film of reconstituted MelB. Difference spectra allow to detect changes in the environment and protonation of some residues, as well as conformational changes of the protein. Deconvolution was applied to the difference spectra to enhance the band sharpness. In the amide I region, we noticed positive and negative bands produced by conformational changes of the protein. A pair of peaks at 1663 cm-1 (negative) and 1658 cm-1 (positive) is assigned to a change in the environment of an alpha helix. A pair of bands at 1700 cm-1 (positive) and 1687 cm-1 (negative) might correspond to conformational changes of unordered structures. Two bands at 1723 cm-1 (negative) and at 1403 cm-1 (positive) are likely to belong to deprotonation of Asp or Glu side chains upon substrate binding. The possibility that these changes occur at the level of the binding sites is considered.

13-55

Domain formation in planar membranes: a fluorescence correlation spectroscopy and monte carlo simulations tudy

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In the recent years it has been found that lateral heterogeneities (rafts) exist in biomembranes. Even in simple pure lipid systems macro- and microdomains can be observed in phase coexistence regions. Coexistence of gel- and fluid domains is also predicted from statistical thermodynamics simulations, and has been visualized by Confocal Fluorescence Microscopy. Gel and fluid domains can influence diffusion processes in membranes. We have investigated diffusion processes of hydrated multilamellar membranes of various phospholipid mixtures supported on a quartz coverslip by Fluorescence Correlation Spectroscopy (FCS). Fluorescence intensity fluctuations, analyzed via a correlation function,

give information on the translational diffusion coefficient $D\tau$ and the diffusion time τ_D. A decrease in temperature results in a lower mean diffusion coefficient caused by the relative increase in the gel domain area with respect to the fluid domains. The shape of the correlation profiles reflects the coexistence of gel and fluid domains. The lateral diffusion is also lowered in the presence of ions, e.g. in the presence of NaCl. We have performed a quantitative comparison of FCS correlation profiles with Monte Carlo simulations (MC). The MC simulations make used of thermodynamical properties of lipid mixtures derived from calorimetric measurements. The MC snapshots show micro- and macrodomains. Since the FCS results are well described by the MC simulations, we arrive at a deeper understanding of the micro-, meso- and nanoscopic details of the membrane structure. This study represents an important step in the understanding of the lateral organization of the lipid membrane, its dynamics and kinetics.

13-56

Lateral Organization of Peptides in Lipid Membranes: A Monte Carlo Simulations and Atomic Force Microscopy Study.

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The analysis of peptide and protein partitioning in lipid membranes is of high relevance for the understanding of biomembrane function. We used statistical thermodynamics analysis to demonstrate the effect of peptide mixing behavior on heat capacity profiles of lipid membranes with the aim to predict peptide aggregation from c_P-profiles. This analysis was applied to interpret calorimetric data on the interaction of the antibiotic peptide gramicidin A with lipid membranes. The shape of the heat capacity profiles was found to be consistent with peptide clustering in both gel and fluid phase. Applying atomic force microscopy, we found gramicidin A aggregates and established a close link between thermodynamics data and microscopic imaging. On the basis of these findings we described the effect of proteins on local fluctuations. It is shown that the elastic properties of the membrane are influenced in the peptide environment. We also applied a similar analysis on the pore forming peptides alamethicin and melittin.

Posters
- Membrane structure and dynamics -

13-57

Structure and interactions in the anomalous swelling regime of phospholipid bilayers

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We have carried out X-ray and neutron diffraction experiments, as a function of temperature, on fully hydrated samples of dimyristoyl phosphatidylcholine (DMPC) bilayers. The data have been analyzed applying a full *q*-range model [1] and show the following: (a) In the vicinity of the L_α to $P_{\beta'}$ transition, we find an anomalous expansion of the water layer of ~ 1.7 Å. (b) The lipid bilayer thickness increases quasi-linearly and is similar to the increase in the lamellar repeat spacing *d* found in dimyristoyl ethanolamine bilayers in the temperature range of T_M to T_M + 13 °C. (c) In contrast to an earlier study, we find no significant changes to the steric size of the phosphatidylcholine headgroup. The anomalous increase in *d* is thus dominated by an expansion of the water layer. This expansion is caused by a distinct increase in bilayer fluctuations as revealed by an analysis of the Caille´ parameter. Additional osmotic pressure experiments not only support this notion but have allowed us to further estimate the temperature dependence of both the bilayer bending rigidity, K_c, and the interbilayer compressional parameter, *B*. Both K_c and *B* experience an abrupt decrease on approaching T_M from above, indicative of a "softening" of the bilayers. These observation account for all outstanding issues concerning structure and interactions in the anomalous swelling regime of DMPC multibilayers and possibly of all lipid bilayers exhibiting similar behavior.

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13-58

Membrane charge effects of anti-inflammatory drugs studied by zeta-potential measurements

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Biological membranes are charged due to ionized or polar groups and the resulting surface potential plays a critical role in regulatory processes and affects the conformation and activity of membrane and membrane-bound enzymes. Thus it is crucial to be able to quantify the effect of non-steroidal anti-Inflammatory drugs on the membrane's surface potential.
The present work studied the concentration effects of indomethacin and acemetacin on the surface potentials of EPC liposomes by measuring the values of zeta-potentials (ζ) in the presence of different drug concentrations. The results show that, indomethacin and acemetacin, both bearing negative charge at physiological pH cause strong concentration dependent membrane charging. Data was interpreted in terms of Gouy-Chapman-Stern theory, that enabled to relate ζ with surface potentials. The membrane loading state (number of molecules per unit area) was calculated for the negative and neutral forms of the drugs. An approach was also developed which allows the determination of the maximum number of molecules per unit area and estimate the maximum mole lipid:drug ratio. Furthermore, the concentration profiles for both drugs can be established in terms of distance to the liposome surface.

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13-59

Structural modofications induced by a general anesthetic on dppc membranes

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The structural modifications of the Dipalmitoylphosphatidylcholine organization induced by increasing concentration of the volatile anesthetic Enflurane have been studied. The interaction of Enflurane with DPPC depends on at least two factors: the Enflurane to lipid concentration ratio and the initial organization of the lipids. At 25 degrees Celsius (gel state), the penetration of Enflurane within the lipids induces the apparition of two different mixed Enflurane-lipid phases. At low anesthetic:lipid molar ratio, the smectic distance increases whereas the direction of the chain tilt changes creating a new gel phase. At high ratio, the smectic distance is much smaller than for the pure gel DPPC phase, i.e. 50 angstrom compared to 65 angstrom, the aliphatic chains are perpendicular to the membrane and the fusion temperature of the phase is 33 degrees Celsius. The electron profile of this phase indicates that the lipids are fully interdigitated. At 45 degrees Celsius (fluid state), a new melted phase was found, in which the smectic distance decreased compared to the initial pure fluid DPPC phase. The thermotropic behavior of the mixed phases has also been characterized. Finally, titration curves of Enflurane effect in the mixed lipidic phase has been obtained by using the fluorescent lipid probe Laurdan. For the maximal effects, Enflurane to lipid ratios (M/M), within the membrane, of 1 and 2 have been determined for the new fluid and the interdigitated lamellar phases respectively. All the results taken together allowed to draw a pseudo-binary phase diagram of Enflurane-Dipalmitoylphosphatidyl choline in excess water.

13-60

Conformational fluctuations of active lipid-protein membranes

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Active membranes that we study are lipid bilayers containing also membrane proteins whose functions can be activated by external sources such as light, ATP as well as mechanical forces. Due to the energy input associated with the activation of the functional proteins, such membranes are set in thermodynamic non-equilibrium states rather than equilibrium states and are, as model systems, a step closer to functional biological membranes than membranes in thermodynamic equilibrium. Inspired by the previous work on active membranes [1,2] we have been developing a number of theoretical models for different types of active membranes and investigating the non-equilibrium, conformational fluctuations of the model membranes. In this presentation we will report some of the preliminary results of our theoretical study.

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Posters

- Molecular machines -

14-1

Are slow skeletal muscles really slow ?

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Based on their shortening velocities and ATPase activity rates, myosin heavy chain (MHC) isoforms have been classified into slow and fast isoforms. In adult skeletal muscles, 4 MHC isoforms are expressed: 1 slow (MHC-I) and 3 fast (MHC-IIA, -IIX and -IIB). Here, we have studied the ATPase rates of myofibrils extracted from rabbit muscles containing almost exclusively MHC-I (soleus) or MHC-IIX (psoas). We choose the myofibril as experimental system to study skeletal muscle myosin ATPase activity because it is small enough to be studied by rapid kinetic techniques, and yet, it presents the highly organised structure of the muscle. With it, one can study either the basal activity (that of a muscle in the resting state) or the calcium-activated activity (that of unloaded shortening muscle). We showed that at relatively low temperatures, the soleus myofibrillar ATPase rates are significantly lower than those of psoas myofibrils: at 4 degrees there was a factor of 2.7 in the resting state and of 85 in the unloaded shortening conditions. However, when the temperature was increased, the differences between slow and fast myofibrillar ATPases tended to decrease and at 42 degrees they were identical. Because the body temperature of the rabbit is 39 degrees and since muscle temperature may increase by up to 4 degrees during intense and prolonged exercises, one may ask if slow muscles really have a slow ATPase activity in physiological conditions. CL is grateful to SFB for financial support. BI is recipient of a EU fellowship (HPRN-CT-2000-00091).

14-2

Stochastic theory of actomyosin molecular motor

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In a few recent years serious experimental evidence was acquired indicatiting that the actomyosin motor can be effectively considered a common chemo-chemical machine occurring, however, in a quasicontinuum rather than in a few conformational states distinguished by the conventional kinetics. Slow character of the conformational transition dynamics is the reason why the input and output steady-state fluxes of the machine cannot be described in terms of the conventional rate constants, an a more sophisticated language of the mean first-passage times has to be used. A technique was developed [1] whith the help of which we found relations between basic parameters of the machine's flux-force dependences: the turnover number, the force stalling the motor as well as the degree of coupling between the ATPase and the mechanical cycles, and the mean first-passage times in a random movement between some distinguished conformational substates of the myosin head. The theory proposed, being a generalisation of both the power stroke and the thermal ratchet ideas, is consistent with all presently available data. It explains the recently demonstrated multiple stepping per one ATP molecule hydrolysed by relating it to the long time needed for melting and renewed crystallisation of a particular alpha-helix, essential for coupling of the catalytic and the lever-arm domain motion of the myosin head. [1] M. Kurzynski and P. Chelminiak, J. Statist. Phys. 110, 137-181 (2003).

14-3

Collective effects in molecular motor motility assays

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Single-molecule techniques such as optical tweezers have made it possible to measure step sizes and forces of single molecular motors (Gittes and Schmidt, 1996, Curr. Opin. Solid. St. M. 1: 412). Nevertheless, much remains unknown about the microscopic details of biological force generation. One aspect of motor protein activity is the collective behavior of individual motors in highly organized larger scale organelles and organs, such as the mitotic or meiotic spindle, flagella, or muscle. Much effort has been invested in constructing simplified thermodynamic/stochastic models to describe the dynamics of motors (Julicher et al., 1997, Rev. Mod.Phys. 69: 1269). It appears likely that such models are best suited to explain coarse grained features of motor protein dynamics, such as collective behavior and cooperative effects on a supramolecular scale. We are investigating the effect of mechanically coupling multiple molecular motors, specifically kinesin, ncd and myosin II. We have developed a suspended-filament in vitro motility assay using platforms of micrometer size (approximately 6 micrometer wide and 1.5 micrometer height), made with photolithography techniques. These platforms are used to attach the motors in a well controlled geometry. We present preliminary results using the new assay.

14-4

Minimalistic Modelling of Na+,K+-atpase Steady State Activity

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Three kinetic models will be presented which allow the Na+,K+-ATPase steady-state turnover number to be estimated at given intra- and extracellular concentrations of Na+, K+ and ATP. Based on experimental transient kinetic data, the models simplify the standard Albers-Post scheme by considering three rate-determining steps of the enzyme cycle: E2 -> E1, E1 -> E2P, and E2P -> E2. On the time-scale of these three reactions the faster binding of Na+, K+ and ATP to the enzyme are considered to be in equilibrium. Each model was tested by comparing calculations of the steady state turnover from rate constants and equilibrium constants for the individual partial reactions with published experimental data of the steady-state activity at varying Na+ and K+ concentrations. In order to provide reasonable agreement between the calculations and the experimental data, important factors required in the model were found to be: 1) Na+/K+ competition for cytoplasmic binding sites, and 2) K+-induced stimulation of the reverse reaction E2 -> E1. The models not only allow the estmation of Na+,K+-ATPase activity under given substrate concentrations, they also provides a framework for understanding the mechanisms of regulation of the enzyme’s activity under physiological conditions.

Posters
- Molecular machines -

14-5

Non Proline Induced Kinks in Transmembrane Alpha Helices

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Membrane proteins account for ca. 30% of genes. Although small, the number of X-ray structures for membrane proteins is such that we can start to analyze structural and functional motifs. We have developed an approach based on a combination of structural bioinformatics and molecular dynamics (MD) simulations to explore the conformational dynamics of transmembrane (TM) helices. Initial studies have focused on the presence of a proline residues in TM helices. With its diminished hydrogen bonding potential and bulky, cyclic side chain, proline can induce a potential hinge point in TM helices [1,2]. There are, however, many helices in membrane proteins that exhibit similar distortions to those induced by proline, but in the absence of proline residues. Examples of such helices include: TM2 of rhodopsin [3] (kinked at a Gly-Gly motif); TM-IV of fumarate reductase [4] (kinked near to a Ser-Ser motif); and TM5 of Ca-ATPase [5] (which undergoes conformational change upon Ca2+ binding). We are undertaking extended (multi-nanosecond) MD GROMACS simulations of these TM helices in a hydrophobic solvent, in a membrane-like environment (an octane slab) and in a DMPC bilayer in an attempt to determine whether the helix distortion/kink is an intrinsic function of the helix, or whether it results from packing interactions with neighbouring helices in the intact protein. The results of these simulations will enhance our understanding of the principles underlying the structure and stability of membrane proteins. [1] Sansom, M. S. P. and H. Weinstein (2000). TiPS 21: 445. [2] Bright, J. N. and M. S. P. Sansom (2003). J. Phys. Chem B 107:627-636. [3] Okada, T. et al. (2002), PNAS 99, 9:5982 [4] Lancaster, C. Roy D. et al. (2000)Nature 405:377. [5] Toyoshima, C, et al. (2000). Nature 405:647.

14-6

Investigating myosin-v stepping kinetics with optical tweezers

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Myosin-V is an ATP-dependent motor protein which moves processively along actin-filaments in discrete steps of ~36nm [1,2]. Using a combination of optical tweezers and single molecule fluorescence microscope, we want to gain insight into the chemomechanical cycle of this motor and answer central questions such as degree of processivity, mode of movement, and behavior under load. A new feedback via a piezo table allows us to observe up to 80 steps in one run. [1] M. Rief, R. S. Rock, A. D. Mehta, M. S. Mooseker, R. E. Cheney, J. A. Spudich, Myosin-V stepping kinetics: a molecular model for processivity, PNAS, vol. 97, no. 17, 9482-9486 (2000) [2] A. D. Mehta, R.S. Rock, M. Rief, J. A. Spudich, M. S. Mooseker, R. E. Cheney, Myosin-V is a processive actin-based motor, Nature 400, 590-593 (1999)

14-7

Sinergistic activation of pkc by cofactors in a computational model

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Protein Kinase C (PKC) is a family of serine/threonine kinases grouped in three classes: conventional (cPKC), novel (nPKC) and atypical (aPKC). The cPKC is activated by calcium (Ca2+), diacylglicerol (DAG) phorbol ester, fatty acids and phospholipids. We constructed a computational model of biochemical pathways involved in activation of PKC to study the hypothesis that the enzyme can decode the temporal coordination and the frequencies of its activators. The model simulates 32 binding states of PKC with different combinations of cofactors and several chemical reactions. In this model PKC was activated by different concentrations, number of spikes and duration of stimuli of DAG, phosphatidylserine (PS), Ca2+ and phorbol myristate acetate (PMA). The results showed that the total PKC activity induced by several cofactors simultaneously is higher than the summation of the individual activities induced by each cofactor alone, suggesting a cooperative activation of the enzyme. The results also showed that PKC is sensitive to properties of the stimuli like different temporal coordination and frequency. In conclusion, our model showed emergent properties such as cooperative activation by the cofactors and ability to decode different stimulation protocols that are difficult to be tested experimentally.

14-8

Kinesin motion characterized by interference near-field microscopy

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The Interferential Near Field Microscope (INFM) allows to study the displacement of a unique kinesin along microtubules at high ATP concentration and in absence of external load. In these conditions, this is the first time that the 8 nanometer step of the kinesin has been measured. The principle of the experiment is to detect the light scattering from a small bead attached to the motor going through a periodically modulated light field. We can probe the behaviour of kinesins over a wide range of timescale. With a nanometer precision and a short time resolution (better than microsecond), we have access to the fine details of the step. Now we are going to carry out the study of the motion of other motors, which may not be processive.

Posters
- Molecular machines -

14-9

Instabilities of isotropic solutions of active polar filaments G

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We study the dynamics of an isotropic solution of polar filaments coupled by molecular motors which generate relative motion of the filaments in two and three dimensions. We investigate the stability of the homogeneous state for constant motor concentration taking into account excluded volume and an estimate of entanglement.
At low filament density the system develops a density instability, while at highdensity entanglement drives the instability of orientational fluctuations.

14-10

Mechanism of Ca^{2+} regulation of the molecular motor, *murine* myosin V.

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The "unconventional myosin-V" is a molecular motor involved in cytoplasmic vesicle transport. The heavy chain contains six IQ-motifs, potentially binding apo-calmodulin as light chains. Ca^{2+} can cause dissociation of calmodulin, and inhibits motility. We have quantitated the effects of Ca^{2+} on the stoiochiometry and affinity of interactions of calmodulin and its isolated N- and C- domains with individual myo-V peptides (IQ1 to IQ6) *in vitro.* Dissociation constants range widely, 0.15 nM to 12.5 μM (Ca_4-CaM) and 8.5 nM to 1.55 μM (apo-CaM). Unexpectedly, the effect of Ca^{2+} is to increase affinity for all but two of the peptides (IQ1 & IQ2). Results with the isolated N- and C-terminal domains of calmodulin show their differential affinity in interactions with the IQ-motif, with the N-domain interacting much more weakly than the C-domain. The C-domain binds only 5-25 times more weakly than intact calmodulin. Under suitable conditions (excess peptide), intact calmodulin can bind two IQ-target sequences, one on each domain. Interactions of apo- and Ca_4-calmodulin with double-length, concatenated sequences (e.g., IQ34) can result in complex stoichiometries. Thus, Ca_4-calmodulin forms a high affinity 1:1 complex with IQ34 in a novel mode of interaction, as a "bridged" structure where two calmodulin domains interact with adjacent IQ-motifs. Such a mode of interaction could account for the Ca^{2+}-dependent regulation of motility of myosin-V *in vitro*, by changing the structure of the regulatory complex, and paradoxically causing calmodulin dissociation due to a change in stoichiometry, rather than a Ca^{2+}- dependent reduction in affinity.

14-11

Molecular dynamics of catenanes and molecular machines of nonbiological nature

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Molecular machines mentioned below are meant to be such molecular systems that use for functioning conformational mobility (i.e. hindered rotation around chemical bonds and with molecular construction deformations with formation and breakage of nonvalent bonds). Components of molecular machines move mainly by means of restricted diffusion. As an example of molecular machines of nonbiological nature catenanes (compounds with two interlocked molecular rings) could be proposed. Thus, for example, model catenane ((2)-(cyclo-bis(paraquat-p-phenylene))-(1(2,6)-tetrathiafulvalena-16(1,5)naphtalena-3,6,9,12,15,17,20,23,26,29-decaoxatriacontaphane)-catenane) changes its redox status when electric field is applied, and rotation of the rings takes place. It occurs with fixation at certain moments of the influence. To find out characteristic properties of rings movements under various external conditions computer simulation of the molecule dynamics was carried out. Three cationic forms of the catenane were subjected to geometrical optimization and quantum chemical calculation using software package GAMESS. Molecular dynamics calculation was carried out with use of MoDyP package.

Posters

- Macromolecular assemblies -

15-1

Pressure and temperature behavior of liquid crystalline cholesteryl esters appearing in linear and nonlinear dielectric spectroscopy.

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Cholesteryl esters belong to the most fascinating liquid crystalline compound in which self-ssembling may cause the appearance of the fascinating blue phase on cooling from the isotropic liquid. They are also important from the biotechnological point of view as compounds being the base for cholesterol. In this contribution we present temperature and pressure (up to 1 GPa) studies of cholesteryl oleate and cholesteryl oleyl carbonate based on the broad-band dielectric spectroscopy (BDS) and nonlinear dielectric spectroscopy (NDS). The latter is associated with changes of dielectric permittivity induced by millisecond pulse of a strong electric field. BDS tests gave an insight into the relaxation of a single molecule as well as into transport processes for changing surrounding (in following mesophases) on cooling or pressuring. The application of NDS enabled direct insight into self-assembling mesoscale nanostructures. Noteworthy is the strong evidence of pretransitional behavior on approaching phase transition points, both as a function of temperature and pressure. Studies described above made it possible to obtain pressure-temperature phase diagrams. Previous studies of the authors in this field can be found in [Chem. Phys., 121, 255 (1988); Phys. Rev. E63, 052701 (2001)., Phys. Rev. E65, 041701 (2002)].

15-2

The studies of vitrification process in two polyhydrohy alcohols: d-sorbitol and xylitol at elevated pressure.

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Polyoyls such as xylitol or sorbitol have become more and more important in food and pharmaceutical application [A. Riku et al. Thermochim. Acta, 380, 109 (2001)]. The most important form of polyoyls is the glassy state. In this contribution the process of solidification to glassy state in two polyhydrohy alcohols: D-Sorbitol, Xylitol is investigated under isobaric and isothermal conditions up to 1.8 GPa. Broadband dielectric spectroscopy was employed to probe the dynamics in quoted systems. The dielectric relaxation measurements show that D-Sorbitol and Xylitol have the same (small) coefficient of the glass transition. On the other hand, both activation volume and fragility are found to be an increasing function of molecular size. In case of D-Sorbitol we found that temperature, rather than density, is dominating variable responsible for the super-Arrhenius behavior of temperature dependence of the structural relaxation times. Moreover, our measurements provide new characteristic of the slow secondary relaxation process. As a result we found that the secondary relaxation time evolves with temperature even below apparent splitting/merging point. However, there is no clear effect of compression on the secondary relaxation time in vicinity glass transition. Thus, one can expect that the slow secondary relaxation process in D-Sorbitol and Xylitol is only thermally activated process. Previous studies of the authors in this field can be found in [J. Phys. Chem. B106, 12459, (2002); Phys. Rev. Lett. 88, 095701 (2002)].

15-3

Prodan fluorescence influenced by high density lipoproteins and alcohol.

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PRODAN FLUORESCENCE INFLUENCED BY HIGH DENSITY LIPOPROTEINS AND ALCOHOL The moderate alcohol consumption is associated with a reduced risk of coronary heart diseases (CHD). The plasma lipoproteins are strongly involved in pathogenesis of atherosclerosis so, it would be of interest to look at their interactions with alcohol. Increase in the level of high-density lipoprotein (HDL) correlated with alcohol consumption might contribute to reduction. However, elevated HDL levels are measured in heavy drinkers, but the antiatherosclerotic behavior of HDL was not effective. Therefore, it would be of interest to study the impact of alcohol concentrations on HDL behavior. We have studied the impact of ethanol concentrations on the structure of two human HDL subfractions, HDL2 and HDL3, particularly on lipid-protein interactions. The alcohol concentrations were chosen to mimic the concentrations in the blood stream. To follow the modulations in the lipid-protein interface induced by alcohol the fluorescence spectroscopy was applied on unlabeled, natural, and on HDLs labeled with membrane probe 6-propionil-2-dimethilaminonaftalen (Prodan) which locates in the head group region of phospholipid near the lipid-water interface. Spectra of Prodan are highly sensitive to the polarity and mobility properties of the environment. The comparison of optical behavior; intensity changes, shift in wave lengths, steady state anisotropy, quenching rates; of intrinsic protein probe, tryptophan, and lipid probe Prodan upon alcohol addition indicated the opening of the protein structure and decrease of the ordering in the lipid moiety of HDL particles. The conformational changes have been confirmed by fluorescence resonance energy transfer measurements (FRET).

15-4

Combined dynamic light scattering and cryo electron microscopic studies on aggregation in supersaturated protein solutions.

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The formation of aggregates during early stages of crystallization has been studied extensively by computer simulations, various scattering techniques and to less extent by direct imaging methods. Still the nature of the structure of aggregates remains obscure. Different aggregates ranging from compact, cristalline spheres [1] or liquid-like droplets [2] to fractal clusters [3] or even planar arrays [4] are considered to be critical for crystal growth. Dynamic light scattering (DLS) monitors the change in size and distribution of aggregates with time. Cryo transmission electron microscopy (cryo-TEM) allows topographical studies at submolecular resolution of protein aggregates while they are still dispersed in solution. The combination of the integrating scattering technique and the direct imaging technique facilitates the interpretation of the calculated radii distributions by taking into account the form of the aggregates seen with cryo-TEM, whereas their incidence is given again by the radii distribution. For studies of aggregation kinetics along this line, lumazine synthase from Bacillus subtilis is an excellent model system due to its size (approx. 16 nm diameter), symmetry and reproducible crystallization behaviour. In the course of our investigations growth of aggregates was followed by DLS. Simultaneously, cryo-TEM samples were taken from the same solution to determine the structure of the scattering particles. By this procedure single protein molecules and loosely bound aggregates (up to 400 nm diameter) were identified and assigned to corresponding radii distributions. [1] A.J. Malkin and A. McPherson (1994), Acta Cryst. D50, 385-395. [2] P.R. ten Wolde and D. Frenkel (1999), Theor. Chem. Acc. 101, 205-208. [3] Y. Georgalis et al. (1999), J. Am. Chem. Soc. 121, 1627-1635 [4] S.-T Yau and P.G. Vekilov (2001), J. Am. Chem. Soc. 123, 1080-1089

Posters
- Macromolecular assemblies -

15-5

Scaling up of actin filament stiffness by chirality frustation

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We prepared complexes self-assembled from of lipids and monomeric actin. Two different phases were obtained and observed by optical microscopies, freeze-fracture electron microscopy, and X-ray scattering. The first phase consisted in soft fibers seen by dark field microscopy, and made of composite actine-membrane sheets. Structural details of the these individual sheets revealed by freeze fracture EM showed corrugation with an oscillation amplitude of 40A, and undulation vectors in the range of 200 angstrom. The second phase was a centimeter long stiff fiber visualized by dark field microscopy and between cross polarizers with a color plate. The 3D supramolecular organization was investigated by small angle X-ray diffraction. The well oriented fiber X-ray pattern could be indexed as a centered rectangular lattice. Diffuse scattering due to actin-actin interactions was observed in a single direction. This suggests that actin-membrane interactions leading to composite sheets yields a large stiffness increase, while macroscopic helicity is still observed. On the contrary, when the chirality is frustrated by 3D ordering in a quasi-infinite stack of membranes, the absence of helical deformations (twist) dramatically increases the macroscopic stiffness up to centimeter long persistence length. This work offers a new standpoint for understanding the mechanical properties of different types of actin assemblies in cells.

15-6

Self-association of peptide filaments into biomimetic nanotubes

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Lanreotide is a synthetic octapeptide, a therapeutic analog of somatostatin, which self-associates into monodisperse and large liquid crystalline nanotubes (24nm of diameter), when mixed with water. Characterization of this system, upon a phase diagram approach and using a variety of techniques including electron microscopy, X-ray scattering, FTIR, FT-Raman and DSC, revealed the formation of soluble aggregates at low peptide concentrations and polydisperse embedded nanotubes at high peptide concentrations. On one side, the soluble aggregates could be identified as soluble filaments, i.e. bilayers of beta-sheets fibers, which exhibit common structural characteristics with the filaments forming the liquid crystalline nanotubes. On the other side, the molecular and supramolecular organizations of Lanreotide molecules into the embedded polydisperse nanotubes could be shown to remain identical to their organizations into the monodisperse nanotubes. These data, added to the delimitation of the domains of existence of the different Lanreotide structures in water, were used to propose a self-association model of Lanreotide filaments into nanotubes. Driving forces and interactions of the Lanreotide self-association could be deduced from this model process and from the structural data. They include hydrophobic effect, hydrogen bonds network, aromatic stacking and electrostatic repulsion. When compared with other peptide fibrils, particularly amyloid fibrils, Lanreotide filaments and nanotubes show common general features, such as the beta-hairpin conformation, the antiparallel beta-sheet networks and the major role of aromatic residues in the nucleation process. The self-association process proposed can therefore be relevant for the general understanding of peptide fibrils formation.

15-7

Structural organization and functional interactions of the ftsz bacterial cell division protein

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We are interested in understanding the influence of factors of the intracellular environment upon the structural organization and functional interactions of the FtsZ protein. FtsZ, bacterial ancestor of tubulin, is a main - and essential -component of the bacterial cell division machinery, which makes it an attractive target of new antibacterial agents. We focus our studies on the effect of two important aspects of the intracellular environment that are certain to affect the structural organization and functional interactions of the FtsZ bacterial cell division protein during the formation of the septal complex: molecular crowding and the membrane surface. In contrast to the experimental solution conditions commonly used in the laboratory, the intracellular environment in which FtsZ is located and function in vivo is highly volume-occupied (crowded), with a total concentration of proteins and nucleic acids on the order of 400 grams per liter. We explore the effect of excluded volume on the energetics, dynamics and structure of polymers formed. We also study the influence of lipid environment on polymer formation. We approach our study through the use of atomic force microscopy, optical biosensor technology, analytical centrifugation and light scattering techniques. We have found that excluded volume affects the stability and aggregation state of FtsZ polymers, and that differently charged lipid surfaces affect polymer structure and their surface affinity. These results are important for the understanding of the behavior of FtsZ in vitro and are of potential relevance for understanding bacterial cell division events.

15-8

Metastable liquid-liquid phase separation in supersaturated lysozyme solutions

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The mechanism of protein nucleation is of high relevance in many fields, from pathological protein deposition in neurodegenerative diseases, to industrial separation processes and crystallization. Experimental and theoretical works suggested that protein crystal nucleation can be affected by the presence of a metastable liquid-liquid (L-L) phase separation, or more specifiacally by critical density fluctuation. We have measured the amplitude and correlation length of local concentration fluctuations by static and dynamic light scattering, in the case of supersaturated solutions of hen egg-white Lysozyme (at pH 4.5 and at different NaCl concentrations, up to 7\%). By extrapolating the critical divergent behaviour of concentration fluctuations vs. temperature, we determined the spinodal line, that is the boundary of thermodynamic instability region. Cloud-point measurements were used to determine L-L phase boundary, consistently with previous work. Our results show that spinodal line shifts towards higher temperatures at increasing salt concentrations. The interesting behavior of concentration fluctuations observed in our experiments offer a deeper insight on salt induced precipitation or crystallization of proteins and it prompts new questions to be addressed both experimentally and theoretically.

Posters
- Macromolecular assemblies -

15-9

Novel architecture of the human pyruvate dehydrogenase complex

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The pyruvate dehydrogenase complex (PDC) is a large, highly ordered multi-enzyme complex that plays an important role in cellular metabolism by linking glycolysis with the TCA cycle. It also serves as a model system for the investigation of protein-protein interactions, enyzme cooperativity and active site coupling. The human complex consists of four different proteins (E1, E2, E3, E3 binding protein), all of which are available as recombinant proteins in Escherichia coli. 60 E2 and 12 E3PB molecules form the icosahedral core which provides the framework for the binding of 30 E1 and 6 E3 molecules. We are investigating the subcomplexes formed by the constituent PDC enzymes and the structural constraints that these impose on catalytic function and enzyme regulation. In particular, we are interested in the formation of "cross-bridges" by E3/E3BP and E2/E1 respectively, whose existence has been indicated by previous experiments using isothermal titration calorimetry, cross-linking studies and native gel electrophoresis. Furthermore, we would like to gain insights into the detailed structural organisation of these subcomplexes using a variety of biophysical techniques including analytical ultracentrifugation *(AUC), surface plasmon resonance (SPR), small-angle X-ray (SAXS) and neutron scattering (SANS), as well as protein crystallography. In addition to studying the subcomplexes formed from individual proteins in vitro we are also investigating the reconstituted, active PDC complex. We gratefully acknowledge the financial support by the BBSCR, UK and the Wellcome Trust.)

15-10

Structural determinants of the full-length hiv-1 tat-pcaf bromodomain complex

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The human immunodeficiency virus (HIV) type 1 transactivator protein Tat is crucial for virus replication since it promotes highly efficient transcriptional elongation from the HIV-1 long terminal repeat. Tat activity is regulated by acetylation although, due to the high flexibility and aggregation properties of the protein, very few is known about structural features that characterize this stage in the viral replication cycle. Based in the recently NMR determined structure of the PCAF Bromodomain complexed with an acetylated Tat peptide (AcK50 Tat 47-54) we constructed and simulated by molecular dynamics techniques a model of the full-length Tat-PCAF Bromodomain complex. Simulations were performed in the multi-ns timescale, in the NPT ensemble using Ewald summation method for long-range electrostatics. The full protein-protein complex reveals extensive hydrophobic contacts between the hydrophobic core domain of Tat and the ZA loop of PCAF Bromodomain increasing the binding affinity of both proteins. A combination of theoretical analysis with experimental data provides new insights on the specific molecular recognition by Bromodomains.

15-11

Supramolecular organization of dense phases of nucleosome core particles : a x-ray diffraction study.

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In eukaryotic chromatin, proteins associate with DNA and condense the molecule into the nucleosomic filament made of the succession of nucleosome core particles linked together by DNA segments. This filament is itself compacted into higher order structures through the association with other proteins, namely H1 histones. A first step in the understanding of the supramolecular organization of this filament and its role in the regulation of DNA expression consists in analyzing the properties of a simplified system, composed of isolated nucleosome core particles (NCP). Multiple dense phases of NCPs were formed in controlled ionic conditions (15 to 160 mM monovalent salt, no divalent ions), under osmotic pressures ranging from 0.47 to 2.35 MPa. We present here the x-ray diffraction analysis of these phases. In the lamello-columnar phase obtained at low salt concentration (below 25mM), NCP stack into columns that align to form bilayers, kept separated from one another by a layer of solvent. NCPs form a monoclinic lattice in the plane of the bilayer. For high salt concentration (above 50mM), NCPs order into either a 2D columnar hexagonal phase or 3D orthorhombic (quasi-hexagonal) crystals. The lamellar and hexagonal (or quasi-hexagonal) organizations coexist in the intermediate salt range; their demixing requires a long time. For an applied pressure P equal to 0.47 MPa, the calculated NCPs concentration ranges from about 280 to 320 mg/ml in the lamello-columar phase to 495-585 mg/ml in the 3D orthorhombic phase. These concentrations cover the concentration of the living cell.

15-12

Harmonic-anharmonic transition in homologues disaccharides/H2O mixtures

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In recent years many efforts have addressed to the understanding of the tools used by organisms to survive under environmental stress conditions. Prominent examples of these survival mechanisms are found in some species of frogs that are able to survive to relatively low temperatures for weeks by means of natural cryoprotective agents that include low-molecular-weight substances such as polyhydric alcohols (commonly glycerol) and sugars (e.g. trehalose), and high-molecular-weight proteins that inhibit ice formation. The bioprotective effects of trehalose have suggested manifold promising applications, such as in preserving vaccines and mammalian cells during drying or cryopreservation, and biological macromolecules like DNA during radiation exposure. Although cryo- and crypto-protectant effectiveness of disaccharides is proved, the underlying molecular mechanisms are not fully clarified. An analysis in terms of elastic scans of the neutron intensity in H2O mixtures of homologues disaccharides such as trehalose, maltose and sucrose as a function of temperature has been carried out. The study provides an effective way for characterizing the dynamical behaviour, furnishing a set of parameters characterizing the flexibility and the rigidity that justifies the better cryptobiotic effect of trehalose in respect to maltose and sucrose. Elastic scans make evident a non-Gaussian behaviour of the intensity profiles which is less marked for trehalose. Furthermore the mean square displacement temperature dependence allows to extract information about the fragility of the investigated systems.

Posters

- Macromolecular assemblies -

15-13

Vibrational properties of cryptoprotectants in water mixtures by inelastic neutron scattering

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New Inelastic Neutron Scattering (INS) results obtained by using the TOSCA spectrometer (ISIS Facility, Rutherford Appleton Laboratory, UK) on disaccharides/H2O mixtures are presented. The comparison among the spectra of trehalose, maltose and sucrose/H2O mixtures, besides evidencing a different destructuring effectiveness on the H2O hydrogen bond network,[1] and hence different cryoprotectant properties, shows a higher "crystallinity" degree for the trehalose/H2O system which accounts for its higher "rigidity".[2,3] This result justifies the better cryptobiotic action of trehalose in respect to maltose and sucrose. [1] C. Branca, S. Magazu', G. Maisano, F. Migliardo, A. K. Soper, Applied Physics A, 74, s450 (2002). [2] C. Branca, S. Magazu', G. Maisano, F. Migliardo, Phys. Rev. B 64, 224204 (2001). [3] C. Branca, S. Magazu', G. Maisano, F. Migliardo, G. Romeo, Philos. Mag. B 82, 347 (2002

15-14

Sans and ins studies on beta-cyclodextrins and hydroxypropyl-beta-cyclodextrins

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This contribution reports experimental neutron scattering results on beta-cyclodextrins and hydroxypropyl-beta-cyclodextrins. Cyclodextrins are cyclic oligomeric compounds which have gained a great importance in many fields of pure research and applied technologies due to their capability of forming inclusion complexes with several organic molecules. The Small Angle Neutron Scattering analysis, carried out by using a core shell cylinder model and the well known Guinier and Zimm procedures, shows that the typical conformation that the investigated system shows in crystalline state is also maintained in aqueous solution. Inelastic Neutron Scattering results evidences the more crystalline character of beta-cyclodextrins in comparison with hydroxypropyl beta-cyclodextrins from the differences in the vibrational spectra of these compounds.

15-15

Neutron scattering study on ethylene glycol and polyethylene glycols

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In recent years new opportunities at the interface of polymer chemistry and biology have been explored at low temperature. As an example, it has been demonstrated that low molecular weight PEGs can be employed in the extractive separation of labile biomolecules such as proteins, offering mild conditions due to the low interfacial tension between the phases allowing small droplet size, large interfacial areas, efficient mixing under very gentle stirring and rapid partition.[1,2] New Inelastic Neutron Scattering findings on Ethylene Glycol and PolyEthylene Glycols at different polymerization degree values are presented. The results evidence a peculiar dependence on the degree of polymerization of the frequencies of the disordered longitudinal acoustic mode which can account for the different bioprotectant effectiveness of PEGs at very low temperature. A comparison with simulation is also performed. [1] G. Coates, LSNM Symposium Abstract, 2001. [2] A. M. Kulkarni, A. P. Chatterjee, K. S. Schweizer, J. Chem. Phys. 113 (2000) 9863-9873.

15-16

Structures of the native and swollen forms of brome mosaic virus determined by cryo-electron microscopy

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The brome mosaic virus is a small RNA plant virus of 270 Angstroms in diameter. As for most RNA plant viruses, the native capsid is stable and compact at low pH while an increase of pH induces a swelling of the capsid that permits the RNA release and thus the infective action of the virus. X-ray crystallography is of little help to determine the structure of the swollen viruses, as the polydispersity of sizes precludes the formation of usable crystals. Conversely, cryo-electron microscopy enables the observation and selection of viral particles corresponding to either state. The structures of the compact and swollen BMV have been determined at a resolution of 25 Angstroms using the icosahedral reconstruction techniques. In each case, the reconstructed volumes show three successive regions: an outer layer corresponding to the protein shell, an inner layer corresponding to the ARN and a central hole. Furthermore, they also reveal that the increase of 8% of the virus diameter is accompanied of the opening of pores in the protein shell and of the redistribution of the ARN. Such observations provide new insight in the way the ARN is released from the BMV capsids.

Posters

- Macromolecular assemblies -

15-17

3d structure of the ligand binding domains of the tumour-associated endo180 lectin using electron microscopy

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Endo180, also known as the urokinase plasminogen activator receptor (uPAR) associated protein (uPARAP), is a member of the mannose receptor family implicated in extracellular matrix remodelling through its interaction with collagens, sugars and uPAR. Endo180 is highly up-regulated in tumour endothelium and in stromal cells associated with breast carcinomas. The extracellular portion of Endo180 contains an N-terminal cysteine-rich domain, a single fibronectin-type II domain and eight C-type lectin-like domains. Each of these types of domains is involved in either protein and/or sugar interactions and their atomic structures have been solved either from a member of the family or a homologous fold. However, little is known as to their 3D arrangement and how this might modulate receptor function. We have purified a soluble version of Endo180 and observed it by single-particle electron microscopy (EM) to obtain a 3D structure of the N-terminal part of the protein at a resolution of 1.7 nm. This volume has been further analysed through the fitting of the atomic structures for each domain into the EM map to describe, for the first time, the interactions between non-adjacent domains in the mannose receptor family.

15-18

Evaluation of cationic detergent-DNA-liposome ternary complexes as gene transfer agents

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Control of the size and stability of DNA complexes are essential for *in vivo* diffusion and gene delivery to cells. Cationic detergents can condense DNA to form small homogeneous but reversible monomolecular complexes. On the other hand, cationic liposomes interact with DNA by forming quasi-irreversible but large aggregates. In this context, ternary complexes consisting of a cationic detergent mixed with plasmid DNA and cationic liposomes were prepared. The formation, stability, size, surface charge, structure, transfection efficiency and cellular trafficking of the ternary complexes were investigated. The ternary complexes were found to exhibit several interesting properties for gene transfer. Indeed, the complexes were found to be small (100 nm) and more homogeneous than the corresponding lipoplexes (250-450 nm). Moreover, the ternary complexes are positively charged and stable in physiological conditions. In addition, from the measurement of the rotational correlation time, rho it was inferred that these complexes exhibited a well established lipid phase internal structure contrasting with the hexagonal structuration of the corresponding lipoplexes. However, though the transfection efficiency of the ternary complexes is greater than that of cationic detergent/DNA complexes, it was significantly lower than that of lipoplexes as assessed from *in vitro* transfection in L929 cells. Confocal microscopy experiments demonstrated that the limited efficiency of the ternary complexes, compared to lipoplexes, is related to their destabilization on the cell surface. This feature may be a consequence of the particular structure adopted by these complexes.

15-19

Dissecting interfaces in protein-protein complexes and in homodimers.

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Two sets of Protein Data Bank entries with 75 protein-protein complexes and 122 homodimeric proteins, were examined for structural features of their interfaces. Each interface was dissected into *recognition patches* by applying a geometric clustering algorithm to atoms in subunit contacts. In protein-protein complexes, a majority of the interfaces bury a surface area *B* in the 'standard' range 1200-2000 $Å^2$ whereas almost all homodimers have larger much interfaces. On average, subunit interfaces are twice larger in homodimers than in complexes, and they are more hydrophobic. 'Standard-size' interfaces generally comprise a single recognition patch on the protein surface. They are common in protease-inhibitor and antigen-antibody complexes, and their formation is compatible with rigid-body association of the component proteins. Larger interfaces are the rule in complexes involved in signalling. They can be split into several patches, and their presence correlates with conformation changes.
Protein-protein interfaces may also be split into a core comprising atoms and residues that are buried in the complex or dimer, and a rim that remains solvent accessible. The core and rim have different amino acid compositions. Whereas the rim composition is very similar to the rest of the protein surface, the core is more hydrophobic, enriched in aliphatic and aromatic side chains and depleted in charged residues other than Arg. In homodimers, the composition of the core resembles the protein interior. These properties relate to the different ways complexes and homodimers assemble, and may help locating surface features involved in protein-protein recognition.

15-20

Nanometric fibres in monolayers of the palmitic acid derivative of the 505-514 fragment of hepatitis G protein. An AFM study.

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Monolayers formed by the amphiphilic lipopeptide, the palmitic acid derivative of the hydrophilic, charged decapeptide, fragment 505-514 of the non-structural protein of the hepatitis G virus (Palm-SAELSMQRRG) were built up in solid – supported monolayers by employing the Langmuir-Blodgett (LB) technique. The structures – nanometric fibres were obtained on mica at different surface pressures and then studied by Atomic Force Microscopy (AFM).
Two kinds of two-dimensional, of a few tens nanometers width fibres were observed. For lower surface pressures shorter fibres of 100-300 nanometers and longer of few micrometers were observed. For higher surface pressures shorter fibres were of 300-1000 nanometers and longer were of few micrometers.

Posters

- Macromolecular assemblies -

15-21

Interaction of porphyrin covalently attached to poly(methacrylic acid) with liposomal membranes

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Recently there is a growing interest in using various phorphyrin dyes as the photosensitizing agents in photodynamic theraphy (PDT) of cancer. It is generally recognized that they have a tendency for the preferential accumulation in solid tumor tissues while introduced to the human body. They can generate short-lived oxidizing species upon irradiation. This leads to the damage of the cell, most likely due to the photosensitized oxidation of lipid cell membrane. Thus the ability of dyes to associate with the membrane surface is an important factor for enhancing the efficiency of PDT.

In this paper we report the results of the model studies on interactions of porphyrin (5-(4-acryloyloxyphenyl)-10,15,20-tritolylporphyrin) covalently attached to poly(methacrylic acid) chain (Po-PMA) with liposomes. The liposomes were prepared from L-α – phosphatidylcholine. Po-PMA is well soluble in water thus aggregation processes are eliminated.

The measurements of fluorescence spectra of liposome/Po-PMA/water system indicated the occurrence of efficient binding of porphyrin with liposome. The binding constants (K_b) were determined using a spectroscopic titration technique. It was found that the values are quite high. As expected they are dependent on pH of aqueous solution and decrease while pH increases. This can be explained, primarily, as resulted from the ionization of PMA polymer chain. These model studies may be useful for the development of well defined and more efficient systems for PDT.

Posters
- Cellular biophysics -

16-1

Thermodynamics of the skeletal muscle contraction

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The results of the thermodynamic studies of the single contraction of the fibre skeletal muscle (m.tibialis of frog), caused by the electrical stimulation (at the frequency of 25 Hz), in the range of temperatures T=(274-278) K are presented. Such thermodynamic parameters as the change of internal energy (deltaU), enthalpy (deltaH) and free energy of Gibbs (deltaG) were calculated in dependence on the fibre skeletal muscle relative strain (epsilon) within the framework of proposed theoretical model. The isothermal change in the entropy (deltaS) was calculated using the experimental (F-L-T) data (?force-length-temperature? phase diagram for the fibre skeletal muscle contraction). In particular, it was found that deltaU=deltaH= -1.55 kT and deltaG= -0.83 kT at epsilon=0.2, and deltaS= -0.72 k (k is a Boltzmann constant). Finally, the value for the Young modulus for the fibre skeletal muscle was estimated: E=(1.2-3.6) MPa (for comparison, E=8 MPa for the rubber). The obtained results were also analyzed in detail within the framework of proposed earlier mechanism of the skeletal muscle contraction [1]. References [1] M.S. Miroshnichenko, M.F. Shuba. Uspekhi Fiziol. Nauk (in Russian), 21, 3 (1990).

16-2

Viability of retinal photoreceptors as tested by their elctrical polarity and distribution of cytosolic calcium

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Living cells often show a structural and functional polarity which is accompanied by electrical polarity due to the assymmetric disposition of ionic pumps. Here we report experimental evidence that retinal photoreceptor cell shows a polar distribution of the electrical charge and free cytosolic Ca2+ along its length. The polar behavior is characteristic to the living cell and vanishes out with the cell functional decay. Both polar Ca2+ and electrical charge distribution can be objectively measured and quantified providing thus a fine test for cell viability. Such a test is required in checking the functional integrity of photoreceptors used in retinal transplant.

16-3

Membrane perturbing activity and multidrug resistance reversal by phenothiazine derivatives

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P-glycoprotein is a transporter of anticancer agents that is commonly over-expressed in in membranes of multidrug resistant cancer cells. Ita drug transporting activity can be inhibited by many compounds, among them phenothiazine derivatives. The molecular mechanism of activity of phenothazine-type multidrug resistance (MDR) modulators still remains unclear. They can inhibit P-glycoprotein by direct binding or by changing the properities of lipid matrix of the membrane in which the protein is embedded. If the latter possiblity were true the anti-MDR potency and membrane perturbing activity of phenothiazines should be correlated. To answer this question we investigated two groups of compounds: phenothiazine maleates and methanesulfonylamides with different ring substituents. Their influence on model membranes was studied by means of fluorescence spectroscopy using probes located in different regions of the bilayer. Microcalorimetry was employed to study the effects of phenothiazine derivatives on thermotropic properties of lipid bilayers. Values of phenothiazines' octanol/water partition coefficients were also calculated. MDR reversal activity was studied by flow cytometric functional test using Rhodamine 123 as P-glycoprotein substrate analogue. All compounds proved to be both active membrane perturbants and effective MDR modulators. The two kinds of activity were positively correlated in case of phenothiazine methanesulfonylamides but not in phenothiazine maleates. This suggests that in spite of closely related chemical structures these two groups of compounds reverse multidrug resistance by dissimilar mechanisms.

16-4

Influence of the laser He - Ne irradiation on platelets aggregation and membrane fluidity in stock suspensions

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Clinical reports signal a pronounced effect of low power laser irradiation (632.8 nm He-Ne laser, red and infrared laser diodes) used additionally or alternatively to the current techniques in curing local infections, inflammations and in microsurgery. They inform us about substantially reduced bleeding, cicatrisation times and improvement of other parameters of local healing. Platelets play a key role in the physiological haemostatic process and in the pathogenesis of thrombosis and cardiovascular disorders. Platelet aggregation has usually been tested by impedance analysis and conventional aggregometry based on light transmission. Platelet aggregometry by the light transmission method demonstrates small detection sensitivity for small aggregates and platelet aggregation is difficult to quantify because of the poor correlation between formation of aggregates and change in light transmission. To overcome these shortcomings, it was proposed to detect platelet aggregates by using light scattering measurements. The capability of the light scattering method to quantify different sizes of aggregates, after stimulation with low concentration of agonists, may facilitate the investigation of the aggregation process. In this study, the effects of low-power He-Ne laser (632.8 nm, 4 mW) irradiation on human blood platelets were investigated, in the presence or not of epinephrine, a well-known aggregating agent. During laser irradiation of blood platelets we noticed changes in platelets aggregation patterns, only when using platelets stimulator. The He-Ne laser irradiation on Platelet Rich Plasma (PRP) obtained from platelet concentrate showed an opposite effect than on PRP separated from fresh blood, emphasizing the importance of pro-aggregating plasmatic factors during this process.

Posters
- Cellular biophysics -

16-5

Peculiarities of single muscle fiber contraction during high-frequency modulated stimulation.

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The tensometric investigations of the skeletal muscle single fibers contraction dynamics of m.tibialis of Rana temporaria frog were carried out under the isometric condition. The dynamic processes ?external load ? force?, developed by muscular fiber under the contractions caused by a change in the frequency of the modulated stimulation, which exceeds physiological level, were investigated in detail. The decrease of the additional muscular force jump in the fore-tetanic region with an increase in the frequency of the stimulating signal was observed during the two-phase stimulation with the frequency of 70 Hz and the total duration of 6 s. It is shown that the increase of the duration of the stimulation causes changes in the non-stationary properties of muscular fiber. We have investigated hysteresis effect which occurs during muscle fiber working cycle and which are poorly studied. Stimulation by square pulses of 0.2 ms duration with the frequency of 0.5-70 Hz was conditionally separated into two time domains. The first one before and the second one was during tetanic contraction. These processes are non ? linear and have significant asymmetry of the time course ? shortening of the muscle proceeds considerably slower than lengthening.

16-6

The hemolytic and physiological activities of mixtures of some organophosphorous compounds with dichlorophenoxyacetic acid

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The application of chemicals of specific destination in multicomponent mixtures may widen their activity spectrum, especially when they exert their toxicity by different modes of action, and may increase the overall activity in comparison with that of a single compound. Mixture toxicity may be approached by somewhat divergent description, from synergistic to antagonistic cooperation between mixed components. This work contains results of studies on combined toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D)and some new organophosphorous compounds that have been synthesized as potential herbicides. There exists the evidence that toxicity of many compounds of biological activity, organophosphorous compounds including, is connected with their lipophilicity that enables them to incorporate into the lipid phase of cell membrane and thus to start physicochemical changes l4eading to living organism metabolism perturbation and/or death. In this work we have checked if binary mixtures of the compounds studied may exhibit higher physiological and membrane-disrupting activity in comparison with that observed for particular compounds. In order to do that, hemolysis of pig erythrocytes and stability of planar lipid membranes formed from soya-bean lecithin as well as changes in the membrane resting potential and electrolyte efflux from alga cells in the presence of the compounds applied alone and in binary mixtures were studied.The results obtained, depending on mixture used, differed from antagonistic to synergistic type. This work was sponsored by the Polish Research Committee (KBN), grant no 6 P04G 096 21.

16-7

Non-genomic effect of 17-β-estradiol on $[Ca^{2+}]_i$ in the ciliary ganglion neurons of embryonic chicks.

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Estrogens such as 17-β-estradiol, play an important regulatory role in vascular, endocrine and nervous systems, through both their membrane and classic estrogen receptors.
In our work, we have studied the rapid effect of 17-β-estradiol on the intracellular calcium concentration ($[Ca^{2+}]_i$) in isolated embryonic chick ciliary ganglion neurons.
Cells were incubated with a calcium sensitive fluorescent dye and monitored *in vivo* with confocal and conventional fluorescence microscopy. The data revealed a significant decrease caused by 17-β-estradiol on the attained effect on $[Ca^{2+}]_i$ by a depolarizing potassium stimulus.This effect can be seen after only 10 minutes of stimulus, proving that no genomic mechanism is involved. The classic receptor blocker ICI182,870 was used to test the implication of the classic receptors ERα and ERβ̃ The implication of cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG), were analyzed as well, by the inclusion of their inhibitors KT5823, which blocked the estrogen effect and KT5720, which did not cause any variation in the effect. Also, an immunocitochemistry assay was performed to reveal the localization of the receptor in the cell. Finally the participation of nictric oxide synthase (NOS) was tested using its inhibitor L-NAME, which also blocked the estrogen effect.
In conclusion, the non-genomic effect of 17-β-estradiol through a citosolic estrogen receptor is mediated by PKG and not by PKA, and the activation of NOS is necessary for the estradiol effect to take place.
These results establish a new model of estrogen action in the nervous system.

16-8

Angiogenesis process driven by cellular traction fields: critical formation conditions and biomechanical modelling of vascular networks.

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Angiogenesis is the formation of new vessels from a pre-existing endothelial cell vascular network, and is of fundamental importance in understanding such processes as dermal wound healing or tumour vascularisation. In vitro assays have shown that the endothelial cell network formation within biogel was very dependent on experimental parameters (initial concentrations) and on mechanical properties of the biogel (Young's modulus, Poisson ratio and shear and bulk viscosities). Therefore, we have considered angiogenesis as a consequence of the mechanical interactions between endothelial cells and extracellular matrix, which both influence active cell migration -haptotaxis- and cellular traction forces. We have developed a biomechanical model accounting for the traction exerted by the cells and the passive mechanical viscoelastic resistance of the matrix to these cellular forces. The model reproduces qualitatively and quantitatively the range of critical values of cell densities and biogel concentration for which a pre-patterning of the biogel with subsequent re-organisation of cells into networks of tube-like structures is observed. A bifurcation analysis is provided to determine critical pattern formation thresholds with specific consideration of changes of extracellular matrix mechanical properties due to cell proteolytic activity. The extended formulation of the model in an hyperelastic context, which will take into account the large biogel deformations during the pattern formation process, will be discussed.

Posters

- Cellular biophysics -

16-9

The role of heat shock protein (Hsp70) in thermoresistance of prostate carcinoma cell line spheroids

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Heat shock protein 70, a protein induced in cells exposed to sublethal heat shock, is present in all living cells and has been highly conserved during evolution. Its function is to protect cells from environmental stress damage by binding to partially denatured proteins, dissociating protein aggregates to regulate the correct folding and to cooperate in transporting newly synthesized polypeptides to the target organelles. The aim of the current study was to determine the role of heat shock protein in the resistance of prostate carcinoma cell line spheroids to hyperthermia. In vitro, the expression of Hsp70 by DU 145 cell line, when cultured as monolayer or multicellular spheroids, was studied using Western blotting and ELISA methods. The level of Hsp70 in spheroid and monolayer cultures remained similar in control (37 degrees of Centigrade cultures) up to 26 days. However, in samples treated with hyperthermia at 43 degrees of Centigrade for 120 min, the spheroid cultures expressed higher level of Hsp70 as compared to monolayer culture. Under similar conditions of heat treatment, the spheroids showed more heat resistance than monolayer cultures as judged by the number of colonies that they formed in suspension cultures. The results suggest that cells cultured in multicellular spheroid models showed more heat resistance as compared to monolayer cultures by producing higher level of Hsp70.

16-10

Fluorescence Microscopy of Polymer-Induced Phase Separation and Divalent Cation-Dependent Compaction and Structural Integrity of Bacterial Nucleoids

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Cellular DNA in prokaryotes is organised as nucleic acid-protein self-assembly, referred to as the nucleoid. The proposal of existence of nucleoid phase separation within cytoplasm is an alternative idea of cytoplasmic organisation to conceptually very attractive model of specific association of nucleoid to bacterial membrane. To understand its nature, our work is focused on weak interactions, coupled with macromolecular crowding, establishing non-membrane bound cytoplasmic organisation. Divalent cation effects on formation of optimum size of thermodynamically stable DNA condensates are emphasized. The effect of excluded volume in solutions with high macromolecular concentrations (macromolecular crowding) upon self-association pattern of reactions is presented in the light of lyotropic liquid crystal theory. Within this respect, interactions between segments of plectonemically supercoiled DNA were described by the second virial approximation. Gel electrophoretic analysis showed that structural results of plasmid configuration of different E. coli strains are in agreement with linking number distributions. Free energy responsible for the DNA packaging acts via decreasing writhing number. Fluorescence microscopy was used for direct visualisation of nucleoids, both in intact cells, and following their electrolysis, after staining with DAPI (4',6-diamidino-2-phenylindole dihydrochloride). Microscopic images correlated well with physicochemical relationships between compaction state, cation concentration and valency, thus indicating an electrostatic mechanism of in vivo bacterial DNA packaging.

16-11

Biochemical characterisation of skin tissue as examined by infrared microspectroscopy

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The structure and biochemical composition of the skin has large impacts on a number of physiological relevant processes [1]. The outermost layer of the skin, the stratum corneum, protects the body from desiccation and thus it is the first permeability barrier of the body. In addition to this, the stratum corneum protects the body against chemical and microbiological impacts. The layer responsible for these effects has a thickness of only 20 microns. The stratum corneum is composed of terminally, differentiated cells (corneocytes), which are surrounded by a continuous lipid phase. The organisation of the corneocytes within the lipid matrix has large impacts on the permeability barrier of the skin [2]. Traditional microscopic methods for the histophathological characterisation of tissues require complicated and time consuming procedures for sample analysis and yield only reduced information concerning the biochemical composition of the sample. The presented method combines the power of infrared spectroscopy for the characterisation of the biochemical components of the sample with spatial resolved information obtained by using a microscope [3]. Evidence is given that the stratum corneum is a rather inhomogeneous tissue [4, 5]. Large lipid domains are observed, especially in the upper layers of stratum corneum. The lateral lipid/protein distribution varies considerably and is a function of the depth of the stratum corneum sample. [1] R. Marks and G. Plewig (1986) Skin models, Springer-Verlag Berlin. [2] A. Schaetzlein, G. Cevc, (1998) British J. Dermatol., 138: 583. [3] W. Huebner et al. Calcif. Tissue Int., 2002 in press. [4] P. Garidel (2002) Phys. Chem. Chem. Phys. (section biophysical chemistry) 4: 5671. [5] P. Garidel (2002) In : Stratum Corneum, edited by R. Marks, J.L. Leveque, R. Voegli, Chapter. 52, pp. 335, Martin Dunitz Ltd.

16-12

DNA electrotransfer : study of factors influencing the repartition of DNA/membrane clusters

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Application of electrical pulses to living cells induces permeabilization of their plasma membrane and can allow gene transfer. The interaction of plasmid DNA with the cell membrane involves both membrane permeabilization and DNA electrophoresis. DNA interacts as clusters only on a limited part of the cell surface facing the cathode. We aim at correlating the number of clusters and the DNA/membrane interaction area to the permeabilized membrane area. We also analyzed how the size and the number of the clusters of DNA vary with the plasmid size, the quantity of DNA incubated or electrical conditions. In this study, a fluorescent microscopy approach was choosen . Gene transfer is a multi-step process which requires interaction of DNA with the cell membrane (during a few minutes after pulses), and then a translocation of DNA and expression of the gene (24 hours later). We have investigated how the early events on cells could be related, in terms of percentage of cells and level of fluorescence, to the later events of expression of the protein encoded by the transgene. Microdomains play key roles in biological membrane fonctions. They are enriched with cholesterol. To study the possible role of microdomains on macromolecule transport, we are now modifying cell membrane composition by depleting membrane with cholesterol and observed DNA/cell interaction. Related reference Golzio M, Teissie J and Rols MP. Direct visualization at the single-cell level of electrically mediated gene delivery. Proc Natl Acad Sci USA. 99 :1292-7 (2002)

Posters
- Cellular biophysics -

16-13

Chloride efflux at the early step of pollen grain germination

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Mature pollen grain is dehydrated and metabolically inactive. Rehydration and activation at the early step of pollen germination precedes pollen tube formation. Earlier we revealed chloride efflux out of tobacco pollen grain during its activation in vitro. Here we answered the questions 1) whether this efflux is important for initiation of germination, 2) what chloride transport proteins of plasma membrane are involved in this efflux, 3) whether chloride efflux out of pollen grain occurs in vivo. Chloride efflux in vitro was measured with chloride selective electrode. Changes in chloride content in pollen grains at the early step of its germination in vivo were detected with electron probe microanalysis on freeze-dried pollinated stigmas. Percentage of pollen grain germination was counted by use of light microscopy. It was shown that inhibition of plasma membrane anion channels by NPPB completely blocked the pollen grain germination in vitro and significantly suppressed chloride efflux. Specific inhibition of antiporters (DIDS, oxonol DiBAC5(4)) as well as simporters (furosemide, bumetanide) did not affect chloride efflux. The data obtained provide evidence that anion channels play an important role in pollen germination in vitro. It was revealed also significant chloride efflux at the early step of pollen germination in vivo. The data as a whole allow suggesting the involvement of chloride transport in regulation of pollen grain germination in vivo as well as in vitro.

16-14

Antioxidant action of flavonoids against the model lipid membranes treated by UV and organic and inorganic lead and tin

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Antioxidant action of flavonoids against the model lipid membrane treated by UV and organic and inorganic lead and tin The research conducted aimed at finding an effective protection against the free-radical action of organic compounds of lead and tin towards phosphatidylcholine (PC) liposome membranes, subjected to factors causing lipid peroxidation. The selected factor was ultraviolet radiation. It was found that in the absence of UV neither the inorganic compounds used nor organic lead and tin compounds did not cause peroxidation of phosphatidylcholine liposomes. In the case of lipid peroxidation induced by UV radiation, the presence of inorganic lead and tin compounds lowered the light induced peroxidation, whereas the presence of organic lead and tin compounds increased peroxidation. In view of the known toxic action of organic lead and tin compounds, we have investigated the possibility of counteracting that phenomenon via chelating those compounds with substances of the flavonoid group. The studied compounds were triphenyllead and triphenyltin chloride; and from the flavonoid group we chose com-pounds: mirycitin, quercetin and kaempherol. Their structure indicates that they can form com-plexes with organic compounds of lead and tin and thus inhibit their harmful effect. All the three flavonoid compounds showed a high antioxidative efficacy with respect to the organic form of tin, whereas in the case of the organolead PC liposome oxidation was most effectively inhibited by mirycitin. We can thus conclude that the protective properties of the flavonoids studied are connected with their chelating ability towards the organometallic compounds stud-ied and also with antiradical ability of the chelates.Work was supported by KBN, grant No. 6PO4G 019 21

16-15

Quantitative Force Measurements of Cell Traction on Artificial 2-D Collagen Based Scaffolds

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In a biomimetic investigation we present a micro-mechanical device enabling the self-assembly of freely suspended quasi two-dimensional collagen networks by arrays of microscopic pillars that mimick biophysical, biochemical, and structural properties of the extracellular site of cells, and which allow to quantify its micro-mechanical properties. Quantification of mechanical properties is based on determination of pillar bending. In combination with cells adhering to the 2-dimensional collagen matrix quantification of stress fields appearing in skin like tissues was possible. The stiffness of the scaffold was varied by the amount of extracellular matrix proteins which assembled on the micro-mechanical device. Thus, it was possible to determine cellular responses to scaffolds of different stiffness.

16-16

Transfer Factor action to the Staphylococcus aureus antigens on the effects of inhibition and excited neurotransmitters in the intestinal smooth muscle

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The Transfer Factor (TF) influence on the contraction-relaxation of the smooth-muscle (SM) preparations of taenia coli was investigated using the tensometric method. It is established that this substance dose-dependently increases the amplitude and duration of single spontaneous contractions, and also the contraction, caused by the depolarization of plasmatic membrane (PM) of the smooth-muscle cells (SMC). TF in the normal Krebs solution always causes a fast increase in both the phasic and the tonic components of the acetylcholine-induced contraction, and also oppresses the Ca2+ release from the IP3-sensitive depot of sarcoplasmatic reticulum. The study of TF influence on the spontaneous contractions of SM under the ATP action showed that the ATP induces the contraction instead of relaxation. Analogous effect was also observed, when ATP was added to the Krebs solution during the tonic component of the acetylcholine-caused contraction took place. It is established that hyperpolarization of PM SMC, caused by ATP action, inverts by TF into depolarization. It was found that substance decreases the amplitude of the junction inhibition potentials (JIP) of PM SM. The preparations did not generate the JIP in the presence of NG-nitro-L-argenin and TF. The results obtained testify that the TF can transfer the ATP inhibition action into the excited one in SMC, and also activate the mechanisms of cholienergic activation in theirs.

Posters

- Cellular biophysics -

16-17

Molecularly defined rigid c(RGDfK) nano-templates regulate cell adhesion by control of aVb3-integrin clustering

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The lack of high resolution bio-patterning methods has prevented direct examination of spatial size ranges that contribute to the process of cell adhesion to tissues or cells on a molecular scale. Studies of the structural arrangements of single ligand bound to integrins in cell membranes, i.e. integrin clustering, demand strict spatial control of cell adhesive areas which bind only one integrin receptor on the nanometer scale. We designed a hexagonally-close packed template for cyclic RGDfK [c(RGDfK)] peptides by self-assembly of diblock copolymers. Nanometer sized cell adhesive dots are positioned with high precision at 28, 58, 73 and 85 nm spacing in hexagonally-close packed patterns over extended glass cover slip areas. The adhesive dots are small enough (< 8 nm) that only a single avb3-integrin bind per dot exclusively. Thus, the template offers a unique rigid c(RGDfK) nanopattern to which only single avb3-integrin receptors can be arranged to obtain adhesion on these nanostructured interfaces. If adhesive dots are separated by more than 73 nm, cell attachment is constricted, as is cell spreading, formation of focal contact clusters, and actin stress fibre formation. Using microstructured nanopattern areas to allow for modulation of local ligand density on a micrometer length scale (second order structure), we demonstrated that this is not due to insufficient ligand density. Rather we attribute this cellular response to restricted avb3-integrin clustering. We found the maximum spacing at which adhesion restriction starts due to integrin clustering failure to be between 58 and 73 nm in MC3T3-Osteoblasts, B16-Melanocytes, and 3T3-Fibroblasts. Since the c(RGDfK) peptide is selective for avb3-integrin occupation, we propose that this length scale is universal in cells which may adhere via avb3-integrins.

16-18

Cell migration in non-differentiated tissues mimics movement in liquids

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Steinberg's Differential Adhesion Hypothesis implies that embryonic tissues, in many respects, mimic the behavior of liquids, but up to date no direct confirmation of this assertion has been provided. We have built a special purpose compression apparatus to test tissue liquidity. Spherical aggregates of Chinese Hamster Ovary cells genetically modified to express N-cadherine were subjected to compression (deformation that increase the surface of the aggregate). Movement of individual cells fluorescently labeled with histone-attached yellow-green fluorescent protein (10% of the total cell number) was followed using confocal microscopy. After the short initial elastic response (related to changes only in cell shape) cells gradually recovered their original shape. The increase in surface area was achieved by the concomitant movement of cells from the interior of the aggregate towards its periphery, similarly to liquids. Cell trajectories were acquired, digitally processed and quantified by calculating the Mean Square Displacements. This method can be used to characterize cell motility under external compressive stress and may be used to assess metastatic potential of malignant cells.

16-19

Effect of stretching on the stiffness of alveolar epithelial cells

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Alveolar epithelial cells in patients subjected to mechanical ventilation undergo considerable stretch. There is little information on how stretching modifies cell mechanical properties. The aim of this work was to measure the changes induced by stretching in the complex elastic modulus of alveolar epithelial cells. We developed an experimental setup to measure cell stiffness during stretching. Cells from a human alveolar epithelial cell line (A549, CCL-185, ATCC, Manassas, VA) were cultured on collagen coated elastic membranes. Homogeneous and biaxial deformations of cell substrate were generated by a vacuum-driven system mounted on an inverted optical microscope. Cell stiffness was measured with an optical magnetic twisting cytometer coupled to the stretching device. Ferrimagnetic beads (4.5 microns) coated with RGD-peptide were bound to cell surface. The beads were magnetized (120 mT) and sinusoidally twisted (5 mT, 1 Hz). The storage (G') and loss (G'') moduli of the cells were computed from the twisting torque and the bead displacement in unstretched baseline and after applying 15% strain (6 membranes). Baseline G' and G'' were 5707 +- 467 Pa and 2255 +- 224 (mean +- SD), respectively. After strain, G' and G'' increased by 28% and 15% respectively. The loss tangent (G''/G') decreased from 0.391 +- 0.044 to 0.354 +- 0.031. All these changes were statistically significant ($p<0.05$, paired t-test). The increase in cell stiffness with stretching could play an important role in disrupting the alveolar epithelial barrier during the inflammatory process associated with mechanical ventilation. Supported in part by SAF2002-03616.

16-20

Measurement of the elastic modulus of soft samples with atomic force microscopy cantilevers

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Accurate estimation of the Young modulus (E) of soft biological samples can be obtained using Atomic Force Microscopy (AFM) cantilevers with a microsphere attached at the tip. However, the large contact surface of these spherical tips does not allow acquisition of high lateral resolution images. On the other hand, the reliability of the common sharp AFM cantilevers for measuring the elastic modulus is poorly defined. The aim of this study was to assess the reliability of cantilevers with sharp pyramidal tips for determining the elastic modulus of soft samples. We compared E of agarose gels (0.3% w/v) with stiffness similar to that of living cells. Measurements were taken with an AFM (Bioscope, Digital Instruments, Santa Barbara, USA) using both spherical (5 microns in diameter) and sharp pyramidal tips. The spring constants (0.01 N/m nominal value) were calibrated from thermal fluctuations. For each cantilever, we acquired 20 deflection-distance (d-z) curves (1 Hz; 1.5 microns amplitude) in three regions of each sample. E was computed by fitting the spherical and pyramidal contact models to the d-z curves with a nonlinear least-squares algorithm. Computed E were 1.11 +- 0.34 kPa and 1.80 +- 0.61 kPa (mean +- SD) for the spherical and pyramidal tips, respectively. The comparable values obtained with both cantilevers indicate that E can be reliably measured with sharp pyramidal tips using the pyramidal contact model. We conclude that mechanical measurements can be obtained in biopolymers and living cells with the cantilevers employed for AFM imaging. Supported in part by SAF2002-03616

Posters
- Cellular biophysics -

16-21

Antioxidant and chelating activity of sulphonic derivative of quercetin

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Quercetin and its glycosides belong to the most frequent flavonoids in the plant world. Quercetin exhibits a high antioxidative activity in vitro. It is barely soluble in water and thus of limited potential application. Instead, the sulphonic derivative of quercetin, quercetin-5'-sulphonic acid (QSA), has a good water solubility and is not toxic. The aim of the study was to determine the antioxidative activity of QSA with respect to phosphatidylcholine liposomes and LDL subjected to peroxidative agents - UV radiation and AAPH - and to compare it with the antioxidative activity of quercetin. The aim of the study was also to determine the efficacy of QSA against the free-radical activity of the organic forms of tin - dichlorodiphenyltin and chlorotriphenyltin - exposed to factors generating free radicals. A high, though by 15-35% lower than quercetin, antioxidative efficacy of QSA with respect to UV-induced oxidation of LDL was found, also in the presence of the organotins. A decidedly weaker, by ca. 70% compared with quercetin, antioxidative activity of QSA was found towards LDL oxidised with AAPH. The percentage of oxidation inhibition caused by QSA with respect to PC membranes oxidised with UV and AAPH was in each case by ca. 20-60% lower than the inhibitive action of quercetin. A possible mechanism of the protective action of QSA and quercetin towards membranes exposed to free-radical action of organotins in the presence of UV radiation was proposed. Work was supported by KBN, grant 6POG 021 19 and grant 308/02/G (Agricultural University)

16-22

Theoretical analysis of epithelial cell bending

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Bending of epithelial sheets plays an important role during the formation of various epithelial tissues. Clearly, the process depends on the mechanical properties of epithelial cells. We present a simple theoretical model of epithelial mechanics. The model assumes that the mechanics of epithelial cells depends primarily on their surface-related properties, such as membrane tension, membrane surface energy and the adhesion between neighboring cells. Possible differences among apical, basal and lateral cell surfaces are taken into account. Epithelial cell shape is described by a simple geometrical model which comprises the three standard deformational modes of planar systems: bending, area expansivity and sheer deformations. The preferred equilibrium cell shape during epithelial deformation can be calculated as the shape with the minimal mechanical energy. The results of the analysis can serve in the interpretation of the epithelial cell shape changes, which, for example, occur during epithelial folding the initial stage of gastrulation.

16-23

Writing on the gemone - high resolution spatial induction of uv photoproducts in cellular dna by 3-photon near infrared absorption

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High-resolution induction of UV photoproducts in cells is a goal of many scientists who study UV DNA damage and repair. We have derived methods whereby near diffraction limited 750nm infra-red laser radiation may be used to induce UV photoproducts in cellular DNA within nanometer dimensions. The use of multiphoton excitation to induce highly localized DNA damage in an individual cell nucleus will provide much greater resolution for studies of DNA repair in the whole cell and facilitate new studies of the interaction of DNA damage and repair with chromatin dynamics. We have defined the parameters of laser radiation (i.e pulse length, intensity and mean power of focused near-infrared radiation) that can be used to induce highly localized DNA damage in a cell with lack of effects from collateral radiations. The damage may be inscribed in the form of a predetermined pattern (even as letters of the alphabet) in the DNA of a cell nucleus [1]. The damage is identified by staining with a specific antibody and the low level of damage has revealed distinctive movement of the DNA lesions in the cell nucleus during the first few hours of repair. Studies using millipore filters with UV radiation to induce localized damage have appeared to illustrate the migration of DNA repair proteins to the sites of damage [2] but movement of the lesions was not detected at the lower resolution used. 1. Meldrum et al (2003) Biophysical J. in press 2. Volker et al, (2001). Molecular Cell 8, 213-224

16-24

Blockade of tetanically evoked calcium and zinc depressions from hippocampal mossy fibers

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Some excitatory pathways of the central nervous system, in particular the hippocampal mossy fiber system, contain large amounts of vesicular zinc. Synaptic transmission between the hippocampal mossy fibers and CA3 pyramidal cells is associated with presynaptic calcium and zinc changes, being the precise role of zinc still unknown. It has been shown that endogenous zinc, assumed to be co-released with glutamate in a calcium dependent way, may interact with numerous ionic channels and receptors. At the mossy fiber synapses, that express an NMDA-independent form of long-term potentiation (LTP), the application of groups of tetani (100 Hz, 1 s), during LTP, evokes posttetanic depressions of single presynaptic calcium and zinc signals and of postsynaptic field potentials. We investigated if these depressions might be caused by tetanically released zinc, combining the use of the fluorescent calcium and zinc indicators Fura-2/AM and N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ), respectively, with the zinc chelators N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) or calcium-ethylenediaminetetraacetic acid (Ca-EDTA). The indicator TSQ, that was first used in histochemical studies, shows an increase in fluorescence, without changes in wavelength, upon zinc binding. The results of the experiments, performed in rat hippocampal slices, show that the permeant zinc chelator TPEN (20 microM) blocks the posttetanic calcium and field potential depressions, being the corresponding zinc depressions abolished by the impermeant chelator Ca-EDTA (2.5 mM).These findings are in agreement with the idea that the observed depressions are mediated by synaptically released zinc causing an inhibitory effect on presynaptic calcium mechanisms. Supported by PRODEP and Plurianual of CNC

Posters
- Cellular biophysics -

16-25

Kinetic and functional properties of the exocrine salivary cell Ca2+, Mg2+-ATPases

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Ca2+ pumps, together with Ca2+ release channels, form unique [Ca2+]i regulatory systems in secretory cells. Plasma membrane Ca2+ -ATPase (PMCA) and the sacro(endo)plasmic reticulum Ca2+ -ATPase (SERCA) together determine resting cytoplasmic Ca2+ concentrations. Because PMCA and SERCA properties in salivary cells membranes are almost unknown, in the present research we studied the characteristics of Ca2+-ATPases in microsomes of rat submandibular salivary gland. The study was performed using post-nucleus, post-mitochondria microsomes obtained by series of consequent centrifugations. Inorganic phosphor (Pi) content, which is liberated during ATP-hydrolysis reaction, was measured using the Fiske-Subarrow method. Total microsomal Ca2+, Mg2+-ATPase activity is characterized by: Pmax - 56 +/- 7.3 mkmole Pi/mg protein, V0 - 8.4 +/- 0.4 mkmole Pi/ min per mg protein and characteristic time of ATP hydrolysis - 6.7 +/- 0.7 min. The apparent Michaelis constants (Km) for ATP is 3.9 +/- 0.5 10-4 M and Hill coefficient (n) = 0.7 +/- 0.1 for PMCA; 4.0 +/- 0.33 10-4 M and n = 0.7 +/- 0.11 for SERCA. Specific activities of PMCA and SERCA at 3 mM of ATP were 440 +/- 69 and 97 +/- 21 mkmole / mg/ h respectively. PMCA and SERCA functioning is precisely regulated by ionized cytoplasmic Ca2+ with Kca 64 +/- 12 M-9, n =1.0 +/- 0.1 and 93 +/- 15 M-9, n = 1.6 +/- 0.1.

16-26

Orientation behavior of retinal photoreceptors in alternative electric fields

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Orientation of photoreceptor rods in alternative fields (AC fields) of various frequencies and intensities was studied in order to correlate the frequency at which orientation changes with dielectric/viability properties of the cell. Orientation of photoreceptor rods in a given medium was shown to be strongly dependent on AC field frequency. On the other hand, the behavior of the cells in AC fields (E = 2.75 V/cm) of different frequencies is strongly influenced by the conductivity of the surrounding medium: - in sucrose 45 % rods are orienting along the field direction at low frequencies (below 450 KHz) while at high frequencies (over 450 KHz) they orient perpendicularly to the field. - in manitol 300 mOsm solution, the cells orientation changes at 230 KHz Using a theoretical model in which the rod shaped photoreceptor cell is approximated by an elongated ellipsoid, it was shown that this behavior is imposed by requirement of minimal interaction energy between the rod cells and electric field. The aim of the present paper is to find a sharp frequency change of cellular orientation depending on dielectrical cell/medium parameters and its correlation with functional and morphological integrity of the living cell.

16-27

Mechanics and force generation of a single cell in presence of cytoskeleton structure modifying bioactive lipids.

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In tissues, constantly regulated processes, such as biochemical and mechanical interactions between a cell and its environment play a major role in differentiation and gene expression of cells. In contrast, cancer cells are able to escape to these regulations and migrate through tissues since their mechanical and adhesion properties change drastically than those of non-tumor cells.
Under physiological conditions, we applied a uniaxial deformation to a single cell adhering between two parallel glass plates (microplates). A precise feedback control allowed for measurements at constant force or constant deformation. Both microplates were functionalized in order to control cell adhesion. Thus, we manipulated a single living cell by controlled adhesion and defined external mechanical stress. We evaluated elasticity and viscosity of different cell types, such as rat embryonic fibroblasts (REF 52), human pancreatic cancer cells (Panc-1) and human gastric cancer cells (AGS). In presence of two bioactive lipids, lysophosphalitidic acid (LPA) and sphingosylphosphorylcholine (SPC), we analysed the mechanical response of Panc-1 and AGS cells. LPA and SPC influenced the organisation of actin- or keratin-cytoskeleton. E.g., elasticity of Panc-1 and AGS cells decreased in presence of SPC consolidating biological studies which predicted that SPC could facilitate metastasis.

16-28

External electric pulsed field on the developmental processes in *in vitro* tissue cultures

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We studied the influence of short duration, moderate-voltage pulses on some proceses in Nicotiana tabacum seedlings cultures: the differentiation of adventitious shoots, the elongation of plants in normal growth contitions and the release of intracellular components (ions, macromolecules) in the electroporation medium. Intact tobacco seedlings were subjected to 78 ms rectangular pulses, with field strength varying between 0 and 500 V/cm. After electric treatment, the seedlings were explanted onto specific culture medium or imersed in deionized water. In a first experiment, inoculum final mass and regeneration capacities were significantly enhanced by electric treatment, showing a “window response” with a maximum at 300 V/cm. In the second one, the normal elongatin of the plant characterized by final mass, and length of stems occurred to be negatively influenced after 300 V/cm proving a loss of hydric equilibrium in porated plants. Finaly, the concentations of ions released by plants in electroporation medium increase after 300 V/cm proving the poration of membranes into the plants.

Posters
- Cellular biophysics -

16-29

Survival/proliferation or apoptosis and calcium regulation in energy and/or nutrient restricted human T cells

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An important event that occurs during energy/nutrient restriction is the loss of ionic homeostasis and cell calcium is thought to play a key role in mediating functional failure, cell death, as well as survival/proliferation in various cells. We aimed to investigate the effects of different components of energy/nutrient restriction (glucose deprivation, blockade of glycolysis, blockade of oxydative phosphorylation, hypoxia/anoxia) on cell fate.
We compare the behavior of human peripheral blood lymphocytes and of human T leukemia lymphoblasts (Jurkat), exposed to stress provoked by energy/nutrient restriction of various severity, and allowed to recover in normal culture medium various time periods. Changes in membrane potential, intracellular pH, intracellular calcium levels, as well as the activation of the MAPK/ERK pathway, elicited by receptor stimulation (PARs and/or TCR), by calcium mobilizing agents (SERCA pump blockers or ionophores) and/or by partial or cuasitotal blockade of metabolic pathways were followed up.
The results obtained provide new experimental evidence concerning the complex relationship between changes occurring in intracellular calcium dynamics, stimulation of MAPK cascades and viability/proliferation or apoptosis of human T cells exposed to energy/nutrient restriction-provoked stress of various severity.
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16-30

Survival/apoptosis and calcium regulation during simulated ischemia, as seen in mdck cells

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The molecular mechanisms leading to ischemic damage in various cells, the intracellular pathways involved in cellular responses and adaptation to ischemia are only poorly elucidated. The aim of this study was to test the ischemic tolerance of Madine-Darby canine kidney (MDCK) cells.
Changes in cell viability, membrane potential, intracellular pH, and intracellular calcium levels elicited by calcium ionophores, blockade of SERCA pumps, and/or of different components of simulated ischemia (glucose deprivation, blockade of glycolysis, blockade of oxydative phosphorylation) were assessed by microscopy and wavelength-domain steady-state fluorimetry. Specific inhibitors of different functional membrane protein complexes were also used in order to delineate some characteristics of various components of the complex calcium signal in MDCK cells.
Attributes of proteinase activated receptor signaling, characteristics of ischemia/reperfusion induced complex calcium signal, and its relations to cell survival/apoptosis, as well as effects during chemical ischemic insult of low concentration of thrombin, and of prior mild ischemia applied to cultured cells, are acquainted
Partial finantial support of the Ministry of Education and Research of Romania (CERES 373/2002) is gratefully acknowledged.

16-31

Creep experiments on isolated myoblasts show soft glassy materials behavior

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We report here the results of creep experiments performed on isolated C2.7 cells. Basically, one living cell is streched between two glass microplates, one rigid and the other flexible. The stiffness of the flexible plate is calibrated so one can deduce the force applied to the cell from its deflection. An original optical detection of the flexible plate deflexion combined with a feedback loop acting on the rigid plate displacement allow us to apply controlled constant stresses (between 5 and 500 Pa) and to follow the time evolution of the strain, i.e. to determine the cell creep function. Below a few minutes, cellular response is quite reproducible and the creep function f(t) scales as a power low of the time t (exponent a = 0,24 +/- 0,08). This is the first evidence of such a behavior at the scale of the whole cell, and make the latter mechanically very similar to soft glassy materials as foams, colloidal suspensions or pastes.

16-32

Direct visualisation at the single-cell level of the first steps of gene delivery by electric field : evidence of a competent microdomain for DNA tran

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Electropermeabilization is one of the non-viral methods successfully used to transfer genes into living cells in vitro as in vivo. Although this approach shows promise in field of gene therapy, very few remains known on the basic processes supporting the DNA transfer. We report here, at the single-cell level by using digitized fluorescence microscopy, the early events of DNA transfer in mammalian cells by electric field pulses. Plasmid interaction with the electropermeabilized cell surface results in the formation of cell associated vesicles where the DNA is trapped. These complexes are stable and cannot be destroyed by pulses of reversed polarities. These membrane associated vesicles are formed only when pulses with duration longer than a critical value are applied. These results are consistent with a multi step model of DNA transfer across the membrane and suggest the existence of competent membrane area for DNA transfer. The electrophoretic accumulation of negatively charged DNA against the permeabilized membrane may act by the associated increase in asymetry of surface charges between the two sides of the cell membrane. This may help in the vesiculation which was observed. These results lead to the conclusion that membrane microdomains may play a role in macromolecules transfer across plasma membrane. Related references Golzio M., Teissie J. and Rols M.P. Direct visualization at the single-cell level of electrically mediated gene delivery. Proc Natl Acad Sci U S A. 99(3):1292-7 (2002)

Posters
- Cellular biophysics -

16-33

Giant vesicles and red blood cells in shear flow. Role of the elasticity of the membrane skeleton

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The motion of soft shells in shear flow is of biological importance since many cells (cancerous cells, leukocytes, red blood cells (RBC)) are submitted to strong shears in the blood vessels. The role of cell deformability has not been quantitatively studied yet although it is of undeniable relevancy to understand mobility, binding and rolling of cells on vascular walls. We quantitatively characterized the behaviour of giant lipid vesicles and RBC in a linear shear flow by optical microscopy. The tank-treading motion (fixed cell orientation), the tumbling motion, the lift force experienced by deformable vesicles and RBC close to a substrate are described as a function of the contrast of viscosity between the inner fluid (haemoglobin for RBC and viscous polymer solution for vesicles) and the outer fluid, and as a function of the distance from the wall. Results are confronted to prediction of models developed for ellipsoids of fixed shape and, recently for deformable soft shells. We showed that the RBC presented a specific behaviour, which originated from the elastic properties of their spectrin skeleton. Firstly, during the tanktreading motion, they oscillate like elastic capsules. Secondly, by varying the shear rate, their motion can pass from the tanktreading to the tumbling regime. These features reveal the existence of a characteristic time involved in the motion, related to the viscoelasticity of the membrane skeleton of the RBC. Finally, we observed the lift motion of vesicles and RBC away from the wall and determined the associated lift force.

16-34

Dynamic regulation of HLA I oligomerization on B cells by exogenous beta-2 microglobulin

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Submicroscopic molecular clusters of class I HLA have been detected by physical techniques (e.g. fluorescence resonance energy transfer, FRET; particle tracking of molecular diffusion, SPT) at the surface of various activated and transformed human cells, including B lymphocytes. We investigated the sensitivity of this homotypic association to exogenous beta2-microglobulin, and the role of free heavy chains (FHC) in class I HLA oligomerization on a human B lymphoblastoid cell line, JY. Scanning Near Field Optical Microscopy (SNOM) and FRET data both demonstrated that FHCs and intact HLA I heterodimers are co-clustered at the cell surface. Culturing the cells with excess beta2-m resulted in a reduced co-clustering and decreased molecular homotypic association, as assessed by FRET. The decreased HLA clustering on JY target cells was accompanied with their reduced susceptibility to specific lysis by allospecific CD8+ cytotoxic T lymphocytes and also provoked significantly weaker T cell activation signals (e.g. lower expression of CD69 activation marker and lower magnitude of TcR down-regulation), than did the untreated B cells. These results together suggest that the actual level of beta2-m available at the cell surface can control CTL activation and the subsequent cytotoxic effector function through regulation of the homotypic HLA-I association. This might be especially important in some inflammatory and autoimmune diseases where elevated serum beta2-m levels are reported.

16-35

Electric-field induced cell deformation is transmitted to neighbouring cells within a confluent monolayer

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When suspended cells are exposed to high frequency electric fields they experience deformation forces that lead to measurable changes in cell shape. In this study we applied a non-invasive impedance technique, referred to as electric cell-substrate impedance sensing (ECIS), to monitor electric field-induced changes in the shape of adherent mammalian cells. The cells were grown as confluent monolayers on small gold-film electrodes (250 microns diameter) deposited on the surface of a plastic substrate. We have applied electric fields of elevated amplitude to cells adherently grown on these electrodes and monitored the associated cell deformation by using impedance readings at properly selected frequencies. By placing a second gold-film electrode rather close (100 to 1000 microns) to the one that carries the elevated electric field, we were able to monitor the transmission of cell deformation to neighbouring cells that were not exposed to the locally applied electric field themselves. Our data suggests that deformation of adherent cells within a confluent monolayer induced by a locally applied electric field is mechanically transmitted to adjacent cells over distances between 200 and 500 microns.

16-36

In vivo and in vitro study of the interaction between the estrogen receptor and putative ERE sequences. Which role for an imperfect ERE ?

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Many important biological processes as differentiation, growth and embryogenesis imply hormone inducible genes. Estrogen activity is mediated by estrogen receptor (ER) that behaves as hormone-inducible transcription factors. The transcription activation is based on the interaction of ER with specific DNA sequences so-called estrogen responsive elements (ERE) without direct estradiol dependence. The ER transactivation mechanisms were investigated, in vivo and in vitro, by performing experiments on the rainbow trout estrogen receptor rtER and both consensus (xlsERE) and putative (rtERE) sequences. To identify and characterize the rtERE in the vitellogenin VIII gene of rainbow trout, beta-galactosidase assays in yeast were performed. rtER expressing plasmids and reporter plasmid bearing either consensus or putative rtERE were co-transfected in S. cerevisiae. To determine the affinity of the ER interaction with ERE sequences, fluorescence anisotropy assays based in solution were performed using recombinant human ER (hER) and fluorescein-labeled ERE sequences. To understand the role played by estradiol in the transcription activation mechanisms, and more precisely the effect of an hormonal stimulation on the rtER localization in living yeasts, both epifluorescence and confocal microscopy attempts are undertaken with a the Dsred-fusioned receptor. Our results have allowed to identify, in vivo, an imperfect ERE sequence in the vitellogenin VIII gene of rainbow trout and also to characterize, in vitro, its affinity as compared to the consensus ERE and non specific sequences. The consequences of a complex decreased lifetime, observed with the imperfect rtERE, will be discussed in terms of physiological relevance for transcription activation.

Posters
- Cellular biophysics -

16-37

A new method for measuring cell viscoelastic properties and the adhesion dynamics of a cell-substrate system

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A new microadhesion experiment is presented, which allows to investigate the interaction dynamics of cell-substrate systems, as well as the cell viscoelastic properties. In the experiment, a functionalised microsphere is brought into contact with cells grown on a glass cover-plate. The test is performed on an inverted microscope equipped with a home-built AFM, which allows to measure forces between the microsphere and the cell. Two experiments are performed: A JKR test and a dynamic separation test. The JKR test is based on cell indentation by the microsphere. By plotting the contact radius (a) versus force (f), a local elastic modulus (E), as well as the adhesion energy, are determined. This method has been adapted further to the case of viscoelastic materials. During dynamic separation, the cell-microsphere adhesion force (f*) is obtained at different peeling velocities (vp) of the cell-microsphere contact area. Results are presented in the case of endothelial cells in contact with beads coated with adhesion proteins.

16-38

Electroporation of adherent cells and electrical in situ monitoring of the morphological cell response

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Electroporation (EP) is defined as the reversible permeabilization of the cellular plasma membranes by exposing cells to strong electric fields (1 to 2 kV/cm) for short time intervals (app. msec). It is generally accepted that hydrophilic pores are formed in the lipid bilayer during the EP-pulse that lead to an tremendous increase in ionic and molecular permeability. EP can not only be applied to suspended but also to adherent cells, in particular when they are grown on a conductive surface that can be used for pulse application. We have combined the experimental requirements for EP of adherent cells with the well-established ECIS technique (electric cell-substrate impedance sensing). ECIS reads the electrical impedance of small, cell-covered gold-film electrodes (250 microns diameter) with weak and non-invasive AC fields. These impedance readings provide information on cell-cell- and cell-substrate-contacts as well as the membrane capacitance. It was the objective of this work to study the efficiency of an EP-induced uptake of extracellular probes and the subsequent recovery of the adherent cells from the EP-pulse. Probe incorporation was determined from the uptake of fluorescence dyes (e.g. Lucifer Yellow and FITC-Dextran) and of small peptides (TRITC-Phalloidin) and proteins (FITC-BSA) from the extracellular space. Cell recovery was monitored with non-invasive ECIS readings. Loading efficiency, cell survival and cell recovery are discussed with respect to the electrical properties of the cells and of the electrodes.

16-39

Adhesion kinetics of functionalized vesicles and mammalian cells

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The suitability of the quartz crystal microbalance technique (QCM) to monitor the formation and modulation of cell-substrate contacts in real time has recently been established. A more detailed analysis of the QCM response when living cells attach and spread on the resonator surfaces is, however, hampered by the chemical and mechanical complexity of cellular systems and the experimental difficulties to control one single parameter of cell-substrate contacts in a predictable way. In this study we made use of liposomes as simple cell models and studied the interactions of these liposomes with the resonator surface. In order to mimic the specific interactions between cell and protein-coated substrate as given in cell culture experiments we incorporated biotin-labeled lipids as receptors in the liposome shell and pre-adsorbed avidin on the resonator surface. The QCM-D technology was applied to monitor the shifts in resonance frequency and energy dissipation during the adsorption of liposomes prepared with increasing amounts of biotin-labeled lipids. We also studied the adsorption kinetics of liposomes doped with biotin moieties that were attached to the lipid core by an alkyl spacer in order to increase the distance between liposome shell and resonator surface. A comparison of this data with the adhesion kinetics of mammalian cells as monitored by QCM-D is presented and discussed. Although the shifts in resonance frequency are very similar for intact liposomes and mammalian cells, the viscous energy dissipation is significantly higher when cells attach and spread on the resonator surface.

16-40

IL-2 and IL-15 receptors sharing lipid rafts are neighbors in similar molecular microenvironments on human T-lymphocytes

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The cytokines interleukin-2 and -15 have common but also contrasting roles in regulating T-cell-mediated immune responses. Functional redundancy is partially explained by sharing the signaling receptor subunits beta and gamma(c) used by both ligands, while the difference in actions may be due to the distinct alpha subunits providing high affinity binding of the appropriate ligand. It is known from T-cell signaling that specific tasks (e.g. peptide recognition by the T-cell receptor and subsequent signaling) are performed by an assembly of molecules including the receptor, co-receptors, kinases, rather than by a single molecule. We raised the question whether the high affinity receptor subunit triplets for IL-2 and IL-15 reside in different molecular environments in the membrane in order to perform distinct tasks, or they just interact differentially with the downstream elements of the signaling cascade in the same microenvironment. We previously showed that on human T lymphoma/leukemia cells the majority of IL-2Ralpha subunits reside in lipid rafts. In Kit 225 FT7.10 cells expressing several hundred thousand copies of the alpha subunit of IL-15R we found by confocal microscopy that the protein is mainly localized in rafts as visualized by the substantial overlap between cholera toxin labeled GM1-rich and IL-15Ralpha-rich membrane domains. Pixel-by-pixel FRET proved that IL-15Ralpha and IL-2Ralpha are in the molecular vicinity of one another. Both IL-2Ralpha and IL-15Ralpha are partially present in a dimeric/oligomeric form, and are associated with MHC-I and MHC-II molecules. Our results imply that the IL-2/IL-15 receptor systems fulfill their diverse roles in similar microenvironments.

Posters
- Cellular biophysics -

16-41

Measuring nucleosome concentrations in living cells with a fluorescence fluctuation microscope

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Although methods for light microscopy of chromatin are well established, there are no quantitative data for nucleosome concentrations in vivo. To establish such a method we used a HeLa clone stably expressing the core histone H2B fused to the enhanced yellow fluorescent protein (H2B-EYFP). Quantitative gel electrophoresis and fluorescence correlation spectroscopy (FCS) of isolated oligonucleosomes show that 5 % of the total H2Bs carry the fluorescent tag and an increased nucleosome repeat length of 204 bp for the fluorescent cells. In vivo, the mobility and distribution of H2B-EYFP were studied with a combination of FCS and confocal imaging. With FCS, concentration and brightness of nascent molecules were measured in the cytoplasm, while in the nucleoplasm, a background of mobile fluorescent histones was determined by continuous photobleaching (CP). Combining these results allows to convert confocal fluorescence images of nuclei into calibrated nucleosome density maps. Absolute nucleosome concentrations in interphase amount up to 260 micromol, with mean values between 100 and 140 micromol, suggesting that a condensation-controlled regulation of site accessibility takes place at length scales well below 200 nm. We note that the method presented here can be applied to any EYFP-tagged fusion protein and may have great potential for a quantitative understanding of molecular thermodynamical properties in living cells. Measuring mobilities and absolute concentrations is important to describe assembly events from transport rates of the monomers over association constants of dynamical binding to density distributions of emerging large scale molecular structures.

16-42

Intracellular H^+/Ca^{2+} signaling in secretion: a boolean intracellular communication protocol?

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In secretory cells, activation of membrane receptors can induce the production of inositol-1,4,5-trisphosphate ($InsP_3$) that can then signal $InsP_3$-receptor channels in the granular membrane turning secretory granules into Ca^{2+} oscillators that deliver periodic trains of Ca^{2+} release to the cytosol, leading to granule transport and exocytosis. Here, we show that $InsP_3$ can also turn mast cell granules into proton oscillators. $InsP_3$-induced intralumenal $[H^+]$ oscillations are ATP-independent, result from H^+/K^+ exchange in the heparin matrix, and produce perigranular pH oscillations with the same frequency, and in-phase with intraluminal $[H^+]$, but out-of-phase with the corresponding perigranular $[Ca^{2+}]$ oscillations. The low pH of the secretory compartment has critical implications in a broad range of intracellular processes. However, the association of proton release with $InsP_3$-induced Ca^{2+} signals, their similar periodic nature, and the sensitivity of important exocytic proteins to the joint action of Ca^{2+} and pH, strongly suggests that granules might encode a combined Ca^{2+}/H^+ intracellular signal. The question of how messages are delivered to specific intracellular targets has been constrained to localization, and/or amplitude/duration or frequency modulation of Ca^{2+} signals. Messages containing conditional Boolean arguments like "if" or "and", requiring more than one instruction for effective readout, still remain vastly unexplored. A combined H^+/Ca^{2+} signal could significantly increase the specificity of the information sent by the granule by transmitting two frequency encoded messages targeted exclusively to proteins like calmodulin, annexins, or syncollin that are both crucial for exocytosis and require specific combinations of $[Ca^{2+}]$ "and" pH for their action.

16-43

Advances in delayed luminescence studies in biophysical investigation

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The low-level luminescence emitted from biological systems on being illuminated, that lasts long time after the stimulating light has been switched off, is termed Delayed Luminescence (DL).
A relevant experimental work has showed that DL changes are closely connected to the highly organized and hierarchical arrangements of molecules in cells. In unicellular alga *Acetabularia acetabulum* (*A.a.*) DL was correlated to the dynamic cytoskeleton organization. In collagen fibers DL was strongly dependent on water content and significantly altered by organic solvents that interact with water.
The idea that the phenomenon can be connected to the existence of collective electron states challenged the authors in applying a Davydov's soliton model to explain DL results from a theoretical point of view. In particular a correlated behaviour of solitons, essential at their relatively high concentration in polypeptide macromolecules, has been considered. Moreover the correlated soliton model has been generalised in order to describe the DL arising from biological systems after their exposition to relatively high dose irradiation. It appeared that the correlated model of the DL provides qualitative and quantitative explanation of the main characteristic features of the DL spectra from *A.a.* Improvement of the model to explain DL response from collagen are in progress.
In the end, our experimental and theoretical results of DL showed that DL response can be used as an intrinsic and non-invasive tool in biological systems. In this respect, UVA-laser induced photon emission measurements in order to detect biophysical changes between carcinogenic and normal mammalian cells have been used.

16-44

Arrays of microfabricated pillars for studying biomimetic models of the actin cortex

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Arrays of microfabricated pillars are constructed to serve as a template for mimicking the actin cortex. The fabrication of this device involves a combination of photolithographic techniques and plasma etching processes. A two-dimensional network of actin filaments, that is pending from the pillar tops, is fabricated. Due to the 3-dimensional template surface interaction of the filaments hanging in between the pillars with substrate surfaces is prevented. This opens new possibilities to study the mechanics of 2-dimensional actin networks as a function of actin-crosslinkers, and the active diffusion of molecular motors operating on pending networks. The behaviour of this actin cortex model will be compared to cortices in living cells.

Posters
- Cellular biophysics -

16-45

Biophysical and molecular characterization of partially differentiated Stem Cells

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Embryonic stem cells display the ability to differentiate in vitro into a variety of cell lineages. Maintenance of the stem-cell phenotype in vitro requires the presence of a feeder layer of fibroblasts or the presence of myeloid Leukaemia Inhibitory Factor (LIF) in the culture medium. Applying a specific differentiation protocol in the mouse embryonic stem line R1, we have obtained a cell line which displays different properties compared to parental R1 cells. The protocol consisted in LIF withdrawal in monolayer cell culture followed by a gating selection system. The selection construction used allows the expression of neomycin resistance under the control of the regulatory regions of the human insulin gene. The cell line obtained is neomycin resistant and the expression level of particular genes is increased respect to R1, including Pdx-1, Pax6 and Isl-1 which are essential transcription factors in pancreatic β-cells and nervous system development. On the other hand the neomycin resistant cell line has different excitatory properties compared to R1. Using the fluorescent Ca^{2+}-sensitive indicator fura2-AM we analyzed the stimuli response of these cells. We observed that neomycin resistant cells show an increase in intracellular Ca^{2+} in the presence of fatty acid free BSA. This result mimics the stimulation pattern observed in isolated astrocytes. Altogether, these results may indicate that the cell line obtained could be considered as a precursor cell line for pancreatic β-cells or astrocyte-like cells.

16-46

Active Application of an Array of Point Like Shear Forces to a Cell Membrane

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Investigation of adaptive viscoelastic properties of cells is closely connected to the understanding of cytoskeletal network structures such as bundled actin filaments called stress fibres and the actin cortex. To measure actively applied arrayed traction forces to a single fibroblast with multi-subcellular resolution, we have constructed a new technique composed of an array of force sensors and a micro positioning system on top of an inverted optical microscope. The force sensor array is made of elastomeric pillars with determined physical parameters and chemically functionalised tops. Its need to be placed perpendicularly to the cell substrate surface requires an accurate positioning with precision less than 500 nm, which we realized by using a piezo controlled tilt stage and an actuator, respectively. After approaching the array to the cell surface, the pillars tops act as local focal adhesion points. Uniaxial translation of the pillar array causes deflection of pillars due to local cell drag forces. Relaxation observation of each individual pillar allowed for measuring local shear forces. The sum of all pillar observations at the same time point realises the evaluation of the relaxation force field surrounding a cell membrane if stimulated actively by point like shear forces.

16-47

Cytotoxicity of retinol and its photoproduct

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Vitamin A or retinol plays an original role in numerous essential biologic processes such as vision, epidermal keratinisation, cellular differentiation… Because of its strong capacity to regenerate the photodamaged skin, retinol became "the vitamin of the ageing". Retinol belongs to the family of retinoid biological compounds including retinal, retinoic acid and palmitate of retinol, all known to be very sensitive to water, air and sunlight. The main source of vitamin A in the skin is food. Another way to supply retinol in the skin is cream application. However, after application on the *stratum corneum* and during the epidermis crossing retinol may be damaged. The present study aims to control the stability of the vitamin A exposed to oxygen and light modelling the exposition of skin in its environment. *Ex-situ* and *in-situ* photooxydation of retinol in creams may be kinetically follows by Raman spectroscopy. The toxicity of retinol is evaluated on cultures of human keratinocytes. The percentage of alive cells is calculated by its enzymatic activities. With these results, we can make a correlation between the kinetics of appearance of some photoproducts at the expense of the decrease of retinol and the cellular death.

16-48

Cortical microtubules in plant cells benchmarking the dynamic spring

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The dynamic spring model was introduced by Clive Lloyd and others in the early eighties as an explanation for the transversal arrangement and reorientation process of (bundles of) microtubules in the cortex of interphase plant cells. It hypothesizes that microtubules form dynamic linear bundles which can adjust their lengths by relative sliding through active processes, and coil transversely around the long axis of the cell with a length dependent pitch, that can be regulated by the cell through the strength of the active sliding. This model was never explored quantitatively, and remained for many years without being debated in its consequences. We approach the problem quantitatively through a combination of in vitro experiments, where microtubules are polymerized from nucleation seeds in microfabricated chambers, and analytical calculations, where the polymers are modeled as inextensible filaments with bending elasticity confined on a two dimensional surface. We are able to explore quantitatively the consequences of the model and its links to observable phenomena in the plant cell, namely, microtubule arrangement and reorientation, and to discuss the reciprocal roles of free energy minimization and kinetic constraints in determining the observed configurations in the in vitro experiment. Our most important conclusion is that the dynamic spring model is not able to account generically for the transverse alignment of cortical interphase microtubules.

Posters
- Cellular biophysics -

16-49

Adhesion forces exerted by epithelial cells on microfabricated pillar surfaces

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Micropillars with diameter around 1µm are fabricated using photolithography in PDMS (polydimethylsiloxane) on a specific substrate. Knowing the geometrical parameters of the pillars, we measure forces exerted by cells attached on fibronectin treated posts by following the deflection of the posts. The pure bending of this elastic material gives the local force generated on one micropillar. In particular, we study epithelial cells (MDCK) during the adoption of a motile phenotype. In tissues, cells are normally stationnary and non-migratory. However, under special conditions, they adopt a motile phenotype requiring changes in the actin cytosqueleton and attachment to the surface. It is becoming clear that modulation of actin assembly is an important function of small GTP-ases like ARF6 proteins. The modulation of the cytosqueleton and the role of ARF6 proteins are studied using both fluorescence measurements and mechanical variations of forces developped by cells. This approach gives a better understanding of the mechanisms implied during the phenotype change of MDCK cells.

16-50

Microrheology of the actin cytoskeleton : response to oscillating forces exerted with optical tweezers

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We use optical tweezers to exert calibrated stresses on the actin cytoskeleton of adherent fibroblasts and epithelial alveolar cells. Static and oscillating forces (amplitude up to 200 pN, frequency 0.1 to 30 Hz) are applied to silica beads (diameter 3.5 µm) specifically bound to transmembrane integrins and thus linked to the actin network. In the (quasi) static case, we measure the bead displacement generated by a constant force, and deduce the average elastic modulus E, taking into account the degree of bead immersion into the intracellular medium. In the case of alveolar cells, E is about 120 Pa, ranging from 30 to 260 Pa from one cell to the other. We also observe the free relaxation of the cytoskeleton towards equilibrium after release of the force, and notice that a single time constant cannot be assigned to each relaxation. This suggests that the viscoelastic properties of the cytoskeleton are frequency dependent. We perform dynamical experiments by applying oscillating forces to each cell and measuring its individual complex elastic modulus. Both the elastic and loss moduli increase as the same positive power of the frequency x, in the range 0.15 - 0.25. Their ratio seems to be frequency independant. This rheological behavior is similar to the one observed in soft glassy materials (foams, emulsions, pastes...). We also investigate the temperature dependance of the exponent x.

16-51

Intravesicular matrix of cholinergic synaptic vesicles binds and adsorbs different neurotransmitters

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We have recently shown (PNAS, 2003, 100, 3485-90) that cholinergic synaptic vesicles contain an intravesicular matrix, which bind acetylcholine and ATP by electrostatic interactions. The high concentration in which neurotransmitters are stored inside synaptic vesicles is due to this particular property of the matrix. The molecular structure of the matrix is enriched in sialic acid and sulfated glycosaminglycans like keratan sulfate. Intravesicular matrix was isolated from cholinergic synaptic vesicles, which were obtained by differential centrifugation from the electric organ of Torpedo marmorata, and kept in an isosmotic medium free of small ions such as Na+, K+, Ca2+ and Cl-. The endogenous content of acetylcholine and ATP was released by hypotonic shock, followed by an increase of the ionic strength. Emptied matrices were incubated with dopamine and dialyzed in pure water. A similar protocol was used with glutamate. When refilled matrices were challenged with ionic strength (8 mM, NaCl), a release of dopamine or glutamate was detected. This result suggests that intravesicular matrix do not interact specifically with neurotransmitters, on the contrary is able to fix every kind of electrically charged small neurotransmitters. The specificity for the neurotransmitter stored into a synaptic vesicle would be due to the particular transporter present in the synaptic vesicle membrane. This work is supported by grants from MCyT from Spanish Government, CIRIT from Generalitat de Catalunya and LaMarato de TV3.

16-52

Hemichannels ion flux is regulated by syntaxin 1A

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Syntaxin is one of the components of the SNARE complex governing exocytosis in chemical synapses. The present work deals with the possible regulatory role of Syntaxin in electrical synapses. Xenopus oocytes provide a valuable tool for revealing the gating properties of hemichannels because they constitutively express connexin Cx38. We used the two electrode voltage-clamp system to analyse ion flux through endogenous hemichannels. The activation of this flux is mediated by the reduction of extracellular calcium concentration. Initially, we identified specific ion flux through hemichannels by the use of different agents that block hemichannels: heptanol, octanol and flufenamic acid. In Syntaxin 1A expressing-oocytes a significant reduction in the amplitude of the current activated by lowering extracellular calcium concentration was recorded. On the contrary, in Botulinum Neurotoxin C1 injected-oocytes, which cleaves Syntaxin 1, the amplitude of the current was enhanced. This result demonstrates that Syntaxin regulates the ion flux through hemichannels. In addition, oocytes overexpressing fragments of Syntaxin 1A reduced also the amplitude of hemichannels ion flux. Finally Syntaxin 1A and Munc-18 were co-expressed in Xenopus oocytes at different molar proportions. The amplitude of the ion flux through hemichannels increased with the amount of Munc18 expressed. This result indicates that Munc-18 functionally dislocates Syntaxin and suggest a regulatory role of Syntaxin on functional hemichannels. This work is supported by grants from MCyT from Spanish Government, CIRIT from Generalitat de Catalunya and LaMarato de TV3. AM was a fellow from AECI.

Posters
- Cellular biophysics -

16-53

Investigation of the neuron membrane state changes during neurotransmission

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The changes of neuron's electrical activity and contents of membrane bound Ca^{2+} during thermostimulation was investigated by the microelectrode and microflurimetric microscopy methods. The object of research was the identified Retzius neuron from leech segmental ganglion. The ganglion was extracted with the afferent nerve fibers and skin patches which was innervated by this ganglion. It is shown, that during thermostimulation of the skin patches the rhythmic excitation frequency increases in a case of temperature increasing. This process correlates with the reduction of membrane bound Ca^{2+} level. Returning the system to an initial condition by the restoration of temperature is accompanied by the rhythmic excitation frequency decreasing and increasing of membrane bound Ca^{2+} level. Also, using the Raman spectroscopy, we find out that subcellular membrane viscosity decrease during temperature increasing and correlates with rhythmic excitation frequency. Probably, the changes of subcellular membrane viscosity take part in regulation of rhythmic activity using Ca^{2+}-dependent K^{+}-channels.
The interrelation between the specified events and possible mechanisms of rhythmic excitation modulation is discussed.

16-54

The generalized endotoxic principle

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Bacterial lipopolysaccharides (endotoxins, LPS) belong to the most potent immunostimulators in mammals. The endotoxic activities range from beneficial effects (induction of cytokines such as tumor-necrosis-factorα and interleukins) to pathophysiological ones (e.g., fever, hypotension, and septic shock). The endotoxic principle of LPS is located in its lipid A moiety, which for *Escherichia coli*-type LPS consists of a hexaacylated diphosphoryl diglucosamine backbone. This lipid A adopts a cubic inverted aggregate structure – deduced from synchrotron radiation X-ray diffraction measurements - from which a conical shape of the molecule can be deduced, whereas the tetraacyl lipid A precursor IVa adopts a cylindrical shape and is endotoxically inactive, but may block the action of active LPS, i.e., represents an antagonist. We hypothesize that non-lipid A amphiphiles with similar physicochemical properties of amphiphilicity, charge, and shape might mimic the respective lipid A. To test this hypothesis, phospholipid-like amphiphiles with six acyl chains attached to a bisphosphorylated serin-like backbone of varying length replacing the diglucosamine backbone were synthesized. The compound with a short backbone fulfills all criteria of an endotoxic agonist, and that with long backbone fulfills those of an antagonist. This holds true for the human as well as for the murine system. Interestingly, these compounds are inactive in the *Limulus* amebocyte lysate test which is specific for LPS diglucosamine backbone. These results define a general endotoxic principle and, furthermore, provide new insights into an understanding of early steps of endotoxin action.

16-55

Modification of charged and unchargd bilayers fluidity by the presence of a peptide sequence of hepatitis GB virusC

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The modification of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), DPPC/1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-lycerol)] (DPPG) (95%:5%), and DPPC/dipalmitoyl trimethyl amoniumphosphate (DPTAP) (95%:5%) liposomes fluidity by the peptide sequence corresponding to the fragment 125-139 of the protein E2 of Hepatitis GB virus C was measured by fluorescence polarisation. Membrane fluidity was recorded in the temperature interval from 25 to 55ºC using two external probes, 1(4trimethylammoniumphenyl)-6-phenyl-1, 3, 5-hexatriene p-toluene sulfonate (TMA-DPH) and 1,6-diphenyl-1,3,5-hexatriene (DPH). TMA-DPH is a superficial probe while DPH adopts a dipper position in the bilayer. The fluidity of the liposomes depends on temperature, and consequently the probe mobility changes with the fluidity of the liposomes. In previous studies with the external probe $Merocyanine_{540}$ (MC540), we have observed, that the peptide causes a relocation of the probe into the bilayer but the remaining MC540 molecules could have a restricted movement by peptide proximity. To get more information about peptide interaction with bilayers, we have chosen the above described probes that are located at different depth of the membrane. We could observe that the mobility of TMA-DPH was reduced when E2 (125-139) peptide was present in the system, while any change in the mobility of the DPH was observed when the peptide was in the cuvette. With this results, we could said that there is a light interacion between E2 (125-139) and the biliayers, and probably this is only a superficial interaction.

Posters

- Transmembrane signalling -

17-1

Investigating Receptor - G protein Interaction by FRET

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G protein-coupled receptors (GPCRs) constitute one of the major signal-transduction systems in eukaryotic cells, as well as important pharmaceutical targets of increasing interest. The heterotrimeric $G\alpha\beta\gamma$ proteins transmit the receptor signal to the effectors of different signalling pathways. Although signal transduction is a field of intense research the molecular details of the interactions between receptors and their G proteins are not well understood.
As a representative member of the family of GPCRs we have chosen the neurokinin 1 receptor (NK1). NK1 is involved in a variety of different processes, e.g. neural signal transmission, regulation of the intestine, hematopoiesis, perception of pain and neurogenic inflammation. The NK1 receptor couples to the G protein Gq, which activates the IP_3/Calcium pathway.
We use fluorescence resonance energy transfer (FRET) to monitor protein-protein interactions. The NK1 receptor, the G protein subunits $G\alpha q$, $G\beta$ and $G\gamma$ were expressed in HEK cells as labelled fusion proteins with ECFP, EYFP or Citrine, which are suitable couples for FRET measurements. Receptor activation by ligand binding and subsequent activation of G proteins and downstream cellular signalling were observed by fluorescent imaging.

17-2

Signal transduction chain via the human mu-opioid receptor: role of cholesterol and DRMs.

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G protein coupled receptors (GPCR) belong to the protein family of seven transmembrane domains involved in the outside signal transduction to the cell plasma membrane. Two additionnal components are implicated in these processes: an heterotrimeric G protein and an effector protein activated by the G protein in order to promote the cellular response. Recognition and interactions between the partners at the membrane level are necessary for the signal transduction progress and require conformational changes and lateral membrane reorganization. Our work deals with the importance of environmental factors and thus the influence of lipids surroundings on the functionality of the signal transduction chain mediated by the mu-opioid receptor (hMOR) in Chinese hamster ovary (CHO) cells. Incubation of these cells in the presence of cholesterol-free or cholesterol-complexed methyl beta-cyclodextrins allowed us to modulate the membrane cholesterol content. By binding experiments, it was shown that cholesterol depletion leads to a decrease in the high affinity binding sites. This effect, specific for agonist ligand, underlines the role of cholesterol on hMOR which maintain them in a high affinity conformation independently of G proteins. We studied also the implication of Detergent Resistant Membranes (DRMs) in the functionnality of hMOR signal transduction chain. Purification of DRMs from CHO cells has been carried out using classical Triton X100 preparation. Localization of hMOR receptors in DRMs have been investigated and quantifications of ligand binding functions where carried out.Results allowed us to discuss on the organization and dynamics of hMOR signal transduction chain in CHO cells.

17-3

Study of the signaling pathways via the mu-opioid receptor (hMOR) on SH-SY-5Y cell line

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hMOR receptor belongs to the family of G-protein coupled receptor and mediates many effects of opioid compounds including modulation of pain perception and euphoria. Activation of hMOR regulates different classes of G protein causing the activation of multiple G protein signal pathways. It has been shown that lipid environment can modulate activities of hMOR (1). To understand at the atomic level the signal transduction mechanism via hMOR and its modulation by lipid environment which could correspond to a conformational change of the receptor (2), we propose to use complementary biophysical approaches: FRAP, FRET, MSF, SPT using lipid or protein probes. For this, a fusion protein T7-EGFP-hMOR using the signal peptide of the nicotinic receptor alph7 subunit to address the chimeric receptor at the cell surface, was constructed. T7-EGFP-hMOR was transiently expressed in Cos-M6 cells and stable expressed in SH-SY-5Y cells. Confocal images of both expressing cell lines show that the fusion protein was properly localized at the plasma membrane. In addition binding properties for agonist and antagonist were conserved (Diprenorphine 0.3 nM ; DAGO 1.3 nM). In the same way the chimeric receptor maintained its functional properties. Thus T7-EGFP-hMOR, were the N-ter labelled position of EGFP did not alter the pharmacology capacities, expressed in SH-SY-5Y cell line, a more appropriate cell type than CHO or HEK or NRK to perform investigations of hMOR signalisation, have been choose as system model to continue the hMOR study. References: (1) Lagane B et. al. J. Biol. Chem. (2000) 327:33197-33200. (2) Salamon Z et. al. Biophys J. (2000), 79:2463-2474.

17-4

Mechanisms of acetylcholine-induced $[Ca^{2+}]_i$ signaling in rat salivary acinar cells

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Calcium homeostasis and Ca2+ -dependent exocytosis in secretory cells is under strict neuronal control but the mechanisms of such regulation aren't well understood. The principal neuromediator regulating salivary cells functioning is acetylcholine (Ach). Cytoplasmic Ca2+ concentration ([Ca2+]i) was assayed using Fura-2/AM. Application of 5mkM Ach evoked [Ca2+]i transients with the amplitude of 202+/-12 nM (n=54). Due to Ach ability to be endogenously hydrolyzed by acetylholinesterases (AchE) we used AchE inhibitor neostigmine (1mkM). Application of neostigmine together with Ach did not significantly change the amplitude of Ach-induced [Ca2+]i transients (95 +/- 3%, n=7), thus showing the absence of active AchEs in preparation. To further clarify the subsets of AchRs responsible for generation of [Ca2+]i transients we used atropine - potent muscarinic receptor antagonist. Application of Ach in the presence of atropine (10 mkM), gave rise to [Ca2+]i transients with the amplitude of 21+/-5% (n=9) from initial Ach response. Application of cyclopiazonic acid (CPA, 5 mkM) evoked sustained [Ca2+]i rise by 71+/-4 nM (n=10) indicating crucial role of SERCA pump in maintaining residual [Ca2+]i level. Ach-induced [Ca2+]i transients under the action of CPA were reduced by 31+/-5% (n=8) of control. Upon the application of Ach in Ca2+-free extracellular solution the amplitudes of Ach- evoked Ca2+-transients were 44 +/- 3% (n=8) lower then in Ca2+-containing solution. Thus, Ach-induced [Ca2+]i-transients are evoked by activation of Ca2+ release from endoplasmic stores and Ca2+ influx from extracellular space. Hence activation of mAchRs is the main source for Ca2+ elevation in cytoplasm of rat salivary cells.

Posters

- Transmembrane signalling -

17-5

Immobilization of G protein-coupled receptors for pharmacological and functional investigation

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External signals (light, hormones, odorants, drugs) interact with G protein-coupled receptors (GPCRs) and provoke the activation of intracellular heterotrimeric G-proteins. It triggers their dissociation and results in the production of second messengers, due to the interactions of both functional units with the respective enzymes or ion channels. GPCRs represent therefore a major target for therapeutic compounds, but also for molecules involved in smell and taste perception. Here we present generic procedures for immobilization of GPCRs on glass surfaces in order to perform pharmacological and functional studies. We used an optical evanescent wave technique: Total Internal Reflection Fluorescence spectroscopy which offers a selective excitation of the fluorophores at surfaces and therefore on-line detection of molecular interactions occurring at the surface. Native membrane fragments containing the neurokinin-1 receptor protein, grafted with a biotin tag were immobilized to functionalized surfaces with streptavidin, whereas detergent solubilized Kappa-opioid receptor carrying affinity tags was immobilized to surfaces functionalized with antibodies. In both cases, the immobilized GPCRs did not show any alteration of their pharmacology features due to their immobilization. Our approach delivered functionaly active GPCRs immobilized in a uniform orientation by using minute amounts of non purified membrane proteins in native or artificial environments. This will be of general interest for investigating the function of membrane proteins, but also for screening of potential therapeutic compounds.

17-6

Agonist and antagonist binding induce distinct changes in the membrane dynamic organization of the mu opioid receptor

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Membrane dynamic changes accompanying the binding of a peptidic agonist or antagonist ligand to the cloned human mu opioid receptor expressed at the surface of stably transfected fibroblasts have been investigated by Single Particle Tracking. In the absence of ligand, the receptors are found having either a slow directed diffusion mode or a walking confined diffusion mode combining a short term confined diffusion to a long term random walk. While the binding of both ligands modifies markedly but similarly the distribution of the receptors between these two modes, the diffusion coefficients and the domain sizes associated to the walking confined diffusion mode are differentially affected by the presence of each ligand. As this receptor is mainly internalized via clathrin coated pits after agonist binding, we have performed single particle tracking experiments on cells transiently transfected with a clathrin-GFP cDNA to identify the clathrin rich regions. The bound and free receptors with a walking confined diffusion mode never colocalize with clathrin rich regions. For the ligand bound receptors having a slow directed diffusion, they are found mainly colocalized with clathrin rich regions for the agonist but inversely predominantly not colocalized with clathrin rich regions for the antagonist. Our results point out that agonist and antagonist ligands modify in a distinct manner the membrane dynamic organization of this G protein receptor.

Posters

- Ion channels (II) -

18-1

Ion channels in the plasma membrane of mouse embryonic stem cells

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Voltage-gated whole-cell currents have been investigated in undifferentiated mouse embryonic stem cells. Embryonic stem cells (ES) are derived from the inner cell mass of the blastocyst at a stage before it would implant in the uterine wall. The embryonic stem cells are pluripotent and self-replicate and can differentiate into cells from all three germ layers. Whole-cell recordings were obtained from a holding potential of -70mV, depolarizing voltage steps (to potentials where Ca^{++} currents are activated) evoked a rapidly activating, outward current. Voltage-gated whole-cell K^+ currents were shown to increase with progressively longer depolarizing voltage steps, and they could be reversibly abolished by application of 200 μM $CdCl_2$, 2.5mM $CoCl_2$, and 25mM TEA. It was concluded that the depolarization-evoked current was activated by Ca^{++}. Ca^{++}-dependent K+ currents were found in all the identified ES cells tested.Testing the effect of Apamin 100nM on these currents we have found that Apamin blocked a TEA-insensitive component, so, we concluded that this currents are small Ca^{++}-dependent K^+ currents. Finally, voltage-dependent inward Ba^{++} currents were recorded. Outward current was suppressed by internal Cs^+, ATP and external TEA. Inward current activated rapidly and decayed to a variable extent. Currents were due to Ca^{++} channel activation since they were abolished by Nifedipine 5μM. No ATP-dependent K^+ currents were detected in these cells.

18-2

The assessment of a selective inhibition of potassium channels and guanylate cyclase in the relaxation induced by nitric oxide in the human nonpregnant myometrium

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Nitric oxide is a multifunctional molecule that mediates a number of diverse physiological processes. Nitric oxide relaxes smooth muscle cells by stimulation of guanylate cyclase leading to increase of cyclic GMP level, and cyclic GMP – dependent modification of several intracellular processes including activation of potassium channels through cyclic GMP – dependent protein kinase. There are evidences that, in some smooth muscle including myometrium, nitric oxide influences potassium channels independently of cyclic GMP. The aim of our work was to study paths of nitric oxide - induced relaxation of the human nonpregnant myometrium. Nitric oxide inhibited spontaneous contractile activity of human nonpregnant uterus in a dose dependent manner. We have studied the effect of methylene blue and cystamine, the inhibitors of guanylate cyclase and specific blockers of potassium channels (charybdotoxin, apamin and scyllatoxin) on the relaxation of myometrium. Methylene blue and cystamine did not prevent nitric oxide -induced relaxation of the uterine strips. However, we have observed that methylene blue used in different concentrations had direct influence on the spontaneous contractile activity of the myometrial strips. Preincubationt of the strips with charybdotoxin or apamin completely inhibited relaxation caused by nitric oxide while scyllatoxin significantly reduced maximum relaxation of the strips. Our data suggest that exogenous nitric oxide relaxes the human nonpregnant uterus without involvement of cyclic GMP and beside calcium and voltage - dependent charybdotoxin - sensitive potassium channels, apamin - sensitive potassium channels are also present in the human nonpregnant

18-3

The effect of sterol structure on the aggregation of the polyene antibiotic nystatin in lipid vesicles

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Nystatin (Nys) is a polyene macrolide antibiotic that presents antifungal properties. This antibiotic targets the cytoplasmic membranes of fungi where it forms aqueous pores, presumably after interacting selectively with ergosterol (Erg). The toxicity of nystatin is thought to be related to its residual interaction with cholesterol (Chol) in the plasma membrane of mammalian cells. Fluorescence spectroscopy is a very sensitive technique capable of monitoring different oligomeric states of Nys in lipid bilayers [1, 2]. In the presence of Erg- but not Chol-containing POPC lipid vesicles, both the spectral shape and decay kinetics of Nys changed dramatically, reporting the oligomerization of the antibiotic in the membranes. In order to define what the structural requirements are for this sterol-induced effect, the interaction of Nys with brassicasterol (Bras)- and 7-dehydrocholesterol (7-DHC)-containing POPC LUV was studied by fluorescence measurements. Bras has the ring system of Chol and the side chain of Erg and the reverse applies for 7-DHC. Neither of the sterols studied was able to induce the formation of long-lived fluorescent antibiotic oligomers, suggesting that both the ring system and the aliphatic side chain of Erg are important structural characteristics for this effect. An activity assay will also be used as a complementary tool to clarify the mechanism of action of Nys. [1] Coutinho, A., and M. Prieto. 1995. Biophys. J. 69:2541-2557. [2] Coutinho, A., and M. Prieto. 2003. Biophys. J. (in press).

18-4

Modelling of ampicillin binding to bacterial pores

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OmpF is a general diffusion porin from the outer cell wall of Gram-negative bacteria Escherichia coli. It is the preferential pathway of antibiotic like ampicillin (AP). Recently, it was obtained that the interaction of AP with OmpF depends essentially on the sign of the applied voltage as well as on the ion strength of surrounding solution (Nestorovich et al. PNAS USA, 2002, 99, 9789). A thermodynamic model explaining this interaction is presented.

We describe AP as a negatively charged molecule with a nonzero dipole moment. On the other hand, the cluster of Arginines and acidic residues at the constriction of OmpF channel create another intrinsic dipole tilted with respect to OmpF monomer axis. By assuming the AP states at the channel constriction are determined mainly by its electrostatic energy, three main states of the system "AP molecule – OmpF channel " are considered: 1) E-state when AP is near OmpF extracellular side; 2) P-state when AP is near OmpF periplasmic side; and 3) B-state when AP is binded for a given short time at the channel constriction. A thermodynamic model describing these states is developed. On the basis of the model we consider how external factors cause the redistribution of the system AP–OmpF by its states. It is shown that the tilt of the intrinsic OmpF dipole qualitatively explains the asymmetrical shape of the residence time-voltage dependence observed in experiments.

Posters
- Ion channels (II) -

18-5

Modulation of membrane permeability by prion derived peptides

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One of the hypotheses concerning the pathogenic properties of prion protein considers its influence on cellular ion homeostasis. Using the lipid bilayer technique the influence of prion derived peptides on lipid bilayer conductance was characterized. To evaluate the physiological significance and possible pathological functions of the peptides their effect on the membrane potential and respiration rate of hippocampal mitochondria was studied as well. We used a peptide bearing the human prion protein sequence YSNQNNF (PrP [169-175]) and peptide SSQNNF (PrP [170-175]) bearing a naturally occurring mutation N171S linked to schizoaffective diseases in humans. In the present report we show that PrP [170-175] N171S increases the conductance of planar lipid bilayers. Based on the observations of single channel currents recorded in 500/500 mM KCl (cis/trans) we found a single channel conductance of 8 to 26 pS. The native prion peptide PrP [169-175] does not form ion channels in the lipid bilayer. Neither of the peptides changed significantly the membrane potential or respiration rate of isolated rat hippocampal mitochondria. We propose a possible mechanism for channel formation by aggregation of the prion derived peptide.

18-6

Outer membrane protein, ompx, from enterobacter aerogenes: a channel-forming protein.

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The outer membrane protein OmpX (18kDa) from E. aerogenes belongs to a family of proteins described to promote adhesion to and invasion of host cells. This protein seems also implicated in antibiotic resistance mechanisms and was overproduced by resistant clinical isolats of E. aerogenes. With up to 80% identity, OmpX from E. aerogenes should be structurally related to OmpX of E. coli from which the structure was determined by either X-ray diffraction of crystals at 1.9 Å resolution or solution NMR studies. It was shown to consist of an eight-stranded, antiparallel ß-barrel with long extracellular loops. The interior contains a hydrogen-bonding network that includes several charged residues which is against a pore function for OmpX.
We have cloned the OmpX E. aerogenes gene in a plasmid (pBCSK) to induce its overproduction in E. aerogenes ATCC 13048. Level of expression was checked by immunoassays. A downregulation of the major Omp36 porin was detected which could consequently supported low level resistance to ß-lactams. OmpX overexpression was sufficient to perform purification of the protein by preparative electrophoresis followed by electroelution in octyl-polyoxyethylen detergent. Its reconstitution in planar lipid bilayers demonstrates the formation of ion channels with a major conductance level of weak amplitude. Pores did not present voltage closure dependence in the ± 180 mV range as usually observed for trimeric porins, but marked cationic selectivity. This channel-forming activity can support the hypothesis of the OmpX role in functional replacement of nonspecific major porins.

18-7

Molecular and electrophysiological characterisation of ion channels in filamentous fungi

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Although it is well established that ion channels play essential roles in many aspects of animal and plant cell biology, very little is known of their roles in filamentous fungi. The development a laser assisted patch clamp technique for use on fungi has allowed, for the first time, the unambiguous electrophysiological characterisation of ion channels (anion and K^+-selective channels) from the plasma membrane of the filamentous fungi, Aspergillus and Neurospora. Inhibitor studies suggest that they probably have essential roles in filamentous fungal biology.
In an attempt to gain further insights in to the role of ion channel function in fungal physiology, ion channel genes have been identified in the model filamentous fungi, Aspergillus nidulans and Neurospora crassa. Three anion channel genes have been identified in A. nidulans. Studies using heterologous expression and the generation of null mutants reveal a role in cation homeostasis. In Neurospora, the full length sequence of a putative K^+ channel has been cloned. Functional heterologous expression in yeast and patch clamp analysis of transformants was used for the electrophysiological characterisation NcTOKA and reveal a role in both K^+ influx and K^+ efflux.

18-8

Blocking of the cerebellum granular cells voltage-dependent K^+ channels by toxins from mexican, venezuelan and north-african scorpion venoms

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Venomous animals produce an almost limitless array of different protein toxins, many of which interact with voltage-gated ion channels. Some protein toxins bind to the outer vestibule of the ion conduction pore, and they are thought to inhibit the channel by physically occluding ion conduction. Other types of toxins (that are known as gating modifiers) interact with very different regions of voltage-gated ion channels and perturb channel function by a distinct mechanism.
Scorpion venoms are rich sources of peptides which affect membrane permeability for Na^+, K^+, Ca^{++} and Cl^- of excitable and non-excitable cells. These toxins are model for peptides synthesis of new drugs, and they are of special interest to study ion-channel structure.
Our work has been focused on neurotoxic peptides which act specifically as high-affinity K^+channel blockers. About sixty distinct peptides have been isolated from Mexican, Venezuelan and North-African scorpion venoms by a sequence of chromatographic procedures. The toxins screenings has been performed on outward K^+ currents in rat cerebellum granular cells, using the patch-clamp technique in the whole-cell configuration. Some peptides have shown to inhibit with different affinity the K^+ currents of the cultured neurons. We have characterized the interaction of these neuropeptides, and in particular of Td3 from Tityus discrepans, on the two main components of the outward currents: slow and fast activating currents characterized by noninactivating and inactivating kinetics.
The goal is those to find a molecular probe able to dissect selectively with high affinity the two components of the K+ currents.

Posters

- Ion channels (II) -

18-9

Probing the channel-bound shaker b inactivating peptide by stereoisomeric substitution at a strategic tyrosine residue

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A synthetic peptide patterned after the sequence of the inactivating ball domain of the Shaker B K+ channel, the ShB peptide, fully restores fast inactivation in the deletion Shaker B(deltha)6-46 K+ channel, which lacks the constitutive ball domains. On the contrary, a similar peptide in which tyrosine 8 is substituted by the secondary structure-disrupting D-tyrosine stereoisomer does not. This suggests that the stereoisomeric substitution prevents the peptide from adopting a structured conformation when bound to the channel during inactivation. Moreover, characteristic in vitro features of the wild-type ShB peptide such as the marked propensity to adopt an intramolecular beta-hairpin structure when challenged by anionic phospholipid vesicles, a model target mimicking features of the inactivation site in the channel protein, or to insert into their hydrophobic bilayers, are lost in the D tyrosine-containing peptide, whose behaviour is practically identical to that of non-inactivating peptide mutants. These latter findings suggest that the structured conformation required for the peptide to promote channel inactivation, as referred above, is likely to be beta-hairpin.

18-10

Intrinsic tyrosine fluorescence as a tool to study the interaction of the shaker b ball peptide with anionic membranes

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Steady-state and time resolved fluorescence from the single tyrosine in the inactivating peptide ShB of the Shaker B potassium channel (ShB peptide) and in a non-inactivating peptide mutant, ShB-L7E, has been used to characterize their interaction with anionic phospholipid membranes, a model target mimicking features of the inactivation site on the channel protein. Partition coefficients derived from steady-state anisotropy indicate that both peptides show a high affinity for anionic vesicles, being higher in ShB than in ShB-L7E. Moreover, differential quenching by lipophilic spin labeled probes and fluorescence energy transfer using trans-parinaric acid as the acceptor confirm that the ShB peptide inserts deep into the membrane, while the ShB-L7E peptide remains near the membrane surface. The rotational mobility of tyrosine in membrane-embedded ShB, examined from the decay of fluorescence anisotropy, can be described by two different rotational correlation times and a residual constant value. The short correlation time corresponds to fast rotation reporting on local tyrosine mobility. The long rotational correlation time and the high residual anisotropy suggest that the ShB peptide diffuses in a viscous and anisotropic medium compatible with the aliphatic region of a lipid bilayer and support the hypothesis that the peptide inserts into it as a monomer, to configure an intramolecular beta-hairpin structure. Assuming that this hairpin structure behaves like a rigid-body we have estimated its dimensions and rotational dynamics and a model for the peptide inserted into the bilayer has been proposed.

18-11

The role of basolateral Cl- channels in epithelial anion secretion

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Earlier studies suggested the presence of basolateral Cl- channels in several secretory epithelia, but their role is unclear, and they have usually been ignored in the description of transepithelial anion secretion. In this study we used patch clamp and transepithelial measurements to characterize the basolateral Cl- channels in airway and colonic epithelial cells. We found that adenosine A2B receptor agonists activated basolateral Cl- channels in a process that involved A-kinase anchoring proteins (AKAPs). This suggests that basolateral Cl- channels may coexist in a multiprotein complex that includes A2B receptors and protein kinases. Using ion replacement and 36Cl- radioactive flux measurements, we found that inhibition of basolateral Cl- channels switched secretion from Cl- to HCO3-. It is now well established that Cl- ions enter cells via the basolateral Na-K-2Cl cotransporter, whereas HCO3- enters via the electrogenic Na+-HCO3- cotransporter, and both anions are transported across the apical membrane via CFTR channels. Thus our results suggest that the basolateral Cl- channels' purpose is to function as a mechanism for switching between Cl- and HCO3- secretion. When these channels are open, they provide a recycling pathway for Cl- ions that enter the cell via the Na-K-2Cl cotransporter, allowing HCO3- ions to be secreted. When they are closed, transcellular Cl- movement via the basolateral Na-K-2Cl cotransporter and apical CFTR dominates anion secretion. This study not only expands our knowledge about epithelial cell function, but also may have important clinical implications for the treatment of diseases such as Cystic Fibrosis.

18-12

Reversal potential and selectivity of the bacterial porin OMPF

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We present experimental measurements and model calculations of the reversal potential of the channel formed by the bacterial porin OmpF from Escherichia Coli. OmpF is one of the so-called 'wide channels', characterized by a relatively large size, poor selectivity and almost no ion specificity. Although its crystallographic structure has been known for a decade, the connection between atomic structure and physiological properties is not completely understood. Thus, several channel function features still remain unexplored, namely the role of the channel constriction in its selectivity, the physical mechanism responsible for the gating, the channel asymmetry, etc. In a series of experiments we have addressed some of these points trying to establish the relationship between the reversal potential and the selectivity of the channel. OmpF selectivity is found to be strongly dependent on salt concentration. Reversal potential measurements show a substantial asymmetry (ca. 25%) when inverting the salt concentration gradient. This asymmetry related to the directional insertion of the channel is found to be also pH and absolute concentration dependent. Control measurements with neutral and charged host lipids show only slight difference, giving support to the idea that relates selectivity with the fixed charges inside the channel. The available structural information allows for qualitative explanation of the experimental results. Some of the challenges of a complete quantitative description are outlined.

Posters
- Ion channels (II) -

18-13

Patch clamp characterization of calcium channels in early mouse and pig embryos and modelling of cell cycle current dependence

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We report the observation of the detection of a dihydropyridine-sensitive voltage-gated Calcium current in 2-cell mouse embryo recorded in the whole-cell patch-clamp configuration. This Calcium current is, in a 2 cell embryo, a minor fraction of the total Calcium current (30 %) that is due mainly by the T-type component. The ratio between T-type current and this type of current changes during the development from unfertilyzed oocyte to 4-cell embryo. The pharmacological characterization is based on sensitivity to the dihydropyridine Nifedipine and divalent ions such as Cadmium, Nickel. We observed that the amplitude of the L component of the Calcium current is regulated by the cell-cycle progression similarly to the well-known regulation of the T-type component. We measured also the calcium currents in early pig embryos during the progression from 1 cell to 4 cell stage. Our observations suggest that in these embryos, only T-type calcium current is detectable. This finding is based on comparison with the currents observed in mouse embryo and on an apparent insensitivity to dihydropyridine. We performed also single channel recordings in cell-attached configuration from early mouse and pig embryos in order to confirm the whole cell recording and to compare the 2 species single channel kinetics. The observed Calcium currents are fitted by the GHK equation and the resulting fit agree with the experimental data. Finally we built a minimal model based on a system of nonlinear differential equation that reproduce the observed cell cycle oscillations in current amplitude Supported by MIUR GRANT

18-14

A method of evaluation of calcium sources in the human thoracic internal artery contraction - in vitro study

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Like in other types of smooth muscle cells, contraction of vascular smooth muscle requires an increase in cytoplasmic calcium (Ca^{2+}). The elevation of intracellular Ca^{2+} may result from either Ca^{2+} influx through specific membrane channels or release from internal store, primarily sarcoplasmic reticulum (SR) The relative contribution of intracellular and extracellular sources may depend on the tissue and the mode of stimulation. Depolarisation by high concentration of potassium (K^+) opens voltage sensitive Ca^{2+} channels in the plasma membrane causing contraction predominantly by the accelerated entry of extracellular Ca^{2+}. However, contraction induced by interaction of certain agonists such as norepinephrine, with their receptors results in both the release of intracellular Ca^{2+} and extracellular Ca^{2+} influx. The internal thoracic artery (ITA) is a commonly used conduit for myocardial revascularization in surgical treatment of coronary artery disease (CAD). Treatment of (CAD) consists of variety pharmacological agents having different influence on blood vessels. The role of Ca^{2+} released from intracellular stores and the entry of extracellular Ca^{2+} for norepinephrine induced responses in human internal thoracic arteries was investigated. Responses of artery to norepinephrine were recorded under isometric conditions. Before each experiment arterial rings were activated (several times) by 80 mmol/L K^+ administration. The muscle response to K+ depolarisation was designated as a control. After incubation in Ca2+-free solution, responses of arteries to K^+ and to 10-6 mol/L norepinephrine were recorded.

18-15

The antibacterial ceratotoxin a induces ion channels in planar lipid bilayer according to the "barrel stave" model

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Ceratotoxin A, a 36-residue alpha-helical cationic peptide isolated from the Ceratitis capitata, exhibits strong antibacterial activity. To determine its mode of action against bacteria, we investigated the behavior of ceratotoxin A by incorporating it into planar lipid bilayers. Macroscopic measurements displayed highly asymmetric I/V curves and strong voltage and concentration dependence. Single channel conductance experiments showed that ceratotoxin A forms voltage-dependent ion channels in bilayers according to the barrel-stave model. The characteristics of the channel suggest that the most probable conducting aggregates are formed by bundles of five or six helices embedded in the membrane. The narrow hydrophilic sector observed in the helical wheel of the C-terminal part and the presence of six positive charges in the N-terminal one suggest that the C-terminal regions form the pore whereas N-terminal moieties lie on the polar side of the lipid bilayer.

18-16

Drug-protein interaction derived by nmr spectroscopy and conductance measurements on vpu from HIV-1

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Small membrane proteins comprise a highly interesting tool for investigations on membrane association and integration of proteins. Their size makes them accessible to solid phase peptide synthesis with the consequence of achieving enough material for spectroscopic investigations such as e.g. NMR spectroscopy. Conductance measurements with the peptide or protein reconstituted into lipid bilayers is furthermore unrevealing functional data. In this study we are combining these two techniques to explore putative binding sites of a channel blocker of Vpu from HIV-1 Vpu is a short (81 amino acid) membrane protein found in subcellular membranes of HIV-1 infected cells. It comprises a transmembrane (TM) domain on its N terminal side and a larger cytoplasmic domain. The function of Vpu is to facilitate virus release and to initiate the down regulation of the CD4 receptor. The helical TM domain is involved in the first role, whilst for the latter the cytoplasmic domain is found to be responsible. Full length Vpu and solely its TM domain are able to exhibit channel activity when reconstituted into lipid membranes. Derivatives of amiloride have been found to block channel activity of Vpu. We use NMR spectroscopy to determine the binding site of Vpu1-32 (TM domain) when bound to cyclohexamethylene amolride in mimetic micelles (manuscript in preparation). Further investigation of the structural changes in Vpu1-32 induced by the binding of the drug will be studied using multidimensional NMR. Conductance measurements of Vpu reconstituted into lipid bilayers reveal that drug binding is in a dose dependent manner.

Posters
- Ion channels (II) -

18-17

The sodium permeability of voltage-gated hair cell calcium channels of the frog labyrinth

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The selectivity of the Ca channels was studied in isolated frog semicircular canal hair cells recorded under whole-cell or perforated-patch conditions. As found previously, about 60% of the cells exhibited a steady Ca current generated by L- and R-type (termed R2) components, while the remaining ones exhibited an additional R-type current (termed R1) which inactivated in a Ca-dependent manner. Upon reducing external Ca (<10 nM), all cells exhibited a steady current, carried by Na, whose maximal amplitude was 5-6 times larger than the one in normal Ca solution. The I-V curves shifted to the left, the peak Na current being attained at -50 mV. The current activation in Ca was fitted by a single exponential (t1 = 0.7 +/- 0.1 ms), whereas the deactivation was bi-exponential (t2 = 1.2 +/- 0.2, t3 = 9.1 +/- 1.1 ms; n = 22). In 10 nM Ca, both t1 and t2 accelerated by a factor 1.7, whereas t3 was unaffected. In cells exhibiting slow inactivation time constant (>10 ms) of the R1 component, 10 uM nifedipine accelerated inactivation by a factor 1.6, indicating a partial block of the open R1 channel. When Na was the charge carrier, nifedipine was able to partially block also the open R2 channel, the block being more effective at negative potentials, whereas no block was observed in normal Ca solution. In conclusion, in low Ca solution, all three channel types lose their Ca selectivity and they were all affected by nifedipine, although to a different extent.

18-18

The c-terminal of the potassium channel KcsA modulates its structural and functional properties

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The prokaryotic KcsA channel is the first potassium channel whose structure has been determined at high resolution using X-ray diffraction methods. Out of the 160 amino acid residues configuring each of the four identical channel subunits, the reported crystal structure corresponds to the 23-119 sequence, that is, lacking a large portion of the N and C-terminal region. The aim of the present study is to explore the relevance of the C-terminal region of KcsA in determining or modulating structural and/or functional properties of the protein as a bona fide potassium channel. For this purpose, we have produced the KcsA delta125-160, delta120-160 and delta115-160 deletions, along with the entire KcsA and have studied different aspects of their structure and function. Our results show that the yield of protein expression in an E.coli heterologous system and the tetramer to monomer ratio, as seen by SDS-PAGE, decrease as the C-terminal region becomes shorter. Also, the secondary structure studied by FT-IR show profound changes in compactness of the deleted proteins. Moreover, channel opening in response to acidic pH and blockade of potassium currents by the presence of sodium ions was explored. Surprisingly, we obtained an altered selectivity in the deleted channels which, indeed, are permeable to sodium at all voltages studied. This suggest that regions as far from the cytoplasm as the highly conserved channel selectivity filter may be affected allosterically by the C-terminal region.
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18-19

ATP-sensitive potassium channels in renal mitochondria

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Isolated kidney mitochondria swell when incubated in hyposmotic solutions containing K+ salts in a manner inhibited by ATP, ADP, 5-hydroxydecanoate and glybenclamide, and stimulated by GTP and diazoxide. These results suggest the existence of ATP-sensitive K+ channels in these mitochondria, similar to those previously described in heart, liver and brain. Renal mitochondrial ATP-sensitive K+ uptake rates are approximately 140 nmol / (min . mg protein), and promote a slight increase in respiration and decrease in the inner membrane potential. In addition, the activation of ATP-inhibited K+ uptake using diazoxide leads to a decrease of ATP hydrolysis through the reverse activity of the FOF1 ATP synthase when respiration is inhibited. In conclusion, we characterize an ATP-sensitive K+ transport pathway in kidney mitochondria which affects volume, respiration and membrane potential, and may have a role protecting against ATP loss during ischemia.

18-20

Chick RGS2L demonstrates concentration-dependent selectivity for GQ/11 and GI/O pathways that inhibit L-type calcium channels

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In neuronal cells, the influx of calcium ions through voltage-dependent L-type calcium (L) channels couples excitation to multiple cellular functions including neurotransmitter release, regulation of gene expression, dendritic development and synaptic plasticity. In addition to voltage, several neurotransmitters, hormones and cytokines regulate L channel gating via binding to G-protein-coupled receptors and subsequent activation of Gi/o and/or Gq-mediated pathways. Intracellular molecules that modify G protein activity, such as Regulators of G-protein Signaling (RGS) -proteins, are therefore potential candidates for regulating calcium influx through L channels. RGS proteins are GTPase-activating proteins (GAPs) for heterotrimeric G proteins; i.e. they increase the rate of intrinsic GTP hydrolysis of Galpha subunits, promoting the formation of inactive heterotrimers. Here we show that a novel RGS2 splice variant from chick DRG neurons, RGS2L, reduces bradykinin (BK)-mediated inhibition of neuronal L channels and accelerates recovery from inhibition. Chick RGS2 reduces both Gi/o and Gq-mediated components of the inhibition. However, the extent of coupling to each pathway varies with RGS2L concentration. A low concentration of recombinant chick RGS2L (10 nM) preferentially reduces Gq-mediated inhibition, whereas a 100-fold higher concentration attenuates Gq- and Gi/o-mediated components equally. In mammalian systems, significant changes in intracellular RGS2 protein concentrations are likely to result from the up-regulation of RGS2 mRNA following synaptic activity, stress, and drug administration. Our data suggest that factors promoting RGS2L gene induction may regulate calcium influx through L channels by recruiting low-affinity interactions

Posters
- Others -

19-1

BbCI: a cruzipain inhibitor from bauhinia bauhinoides seeds. Cloning, heterologous expression and crystallization

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Bauhinia bauhinoides cruzipain inhibitor (BbCI) is a cysteine proteinase that shows high homology with plant Kunitz-type inhibitors, but it lacks cysteine residues and therefore the disulfide bridges. BbCI is effective in cruzipain inhibition, and thus should be consider in an investigation about potential drugs against Trypanosoma cruzi infection. Besides, the BbCI presents an inhibitory activity to the HLE elastase, which is important in physiopathological process. The cloning of the BbCI gene was realized by RT-PCR. Once confirmed the amplified sequence, internal primers were designed to perform a 5' and 3' RACE. The information generated showed that BbCI appears to be initially synthesized as a prepeptide with the following structure: N-signal (19 residues, mature-peptide (164 residues) and C-terminal-peptide (10 residues). After this, we realized the subcloning of the sequence corresponding to mature chain to the pET28a expression vector. The overexpression in E. coli BL21(DE3) produced the BbCI as a fusion with a histidine tail which allowed its purification by affinity chromatography in Ni-NTA agarose resin. Then, the recombinante BbCI (rBbCI) was released from fusion by thrombin cleavage and gel filtered in Superdex 75. The eluted showed inhibitory activity when was assayed with human elastase. The rBbCI was submitted to measurements of the Light Scattering in order to carry on the initial trials of protein crystallization. Crystals of different morphologies were grown in several conditions and the best was found in 30 percent of PEG 4000, 0.1 M Sodium Acetate at pH 8.6 and 0.2 M Ammonium Acetate. Supported by FAPESP

19-2

How the position and shape of aerial objects viewed binocularly from water change versus head tilting

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It is a well-known phenomenon that looking out of the water with two underwater eyes, both the apparent position and shape of aerial objects are different from the real ones due to the refraction at the water surface. Earlier studies of the refraction-distorted structure of the aerial binocular visual field of underwater observers were restricted to either vertical or horizontal orientation of the line between the two eyes. We investigate here a generalized version of this problem: We calculate the position of the binocular image point of an aerial object point viewed by two arbitrarily positioned underwater eyes, including oblique orientations of the eyes due to head tilting. Assuming that binocular image fusion is performed by appropriate vergent eye movements to bring the object's image onto the foveae, the structure of the aerial binocular visual field is computed and visualized in different ways as a function of the relative positions of the eyes. We show that a revision of certain earlier treatments of the underwater binocular imaging of aerial objects is necessary. We demonstrate that the structure of the aerial binocular visual field of underwater observers distorted by refraction is more complex than it has been thought previously.

19-3

New simulation method for biological macromolecules

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A combination of the analytical solution of the high-frequency harmonic part of the Hamiltonian and the numerical solution of the low-frequency remaining part forms the Split Integration Symplectic Method (SISM) for molecular dynamics (MD) integration. The approach developed here is general and applicable to any biological macromolecular system, but illustrated at present by application to a box of water molecules. The numerical results show that the SISM possesses long term stability, allows an integration time step larger than can be used by other methods of the same order and complexity while the computed IR spectrum matches the experimental one.

19-4

Monolayer cells and three dimensional spheroids of MG-63 osteosarcoma show a different metabolic profile as demonstrated by 1H-NMR studies

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Cultures of cells in monolayer have provided a deeper understanding of the cellular mechanisms responsible for tumor cell behavior while three-dimensional ones (i.e., multicellular spheroids) have permitted the study of cell-cell interactions in a context more closely resembling tumor tissue architecture. The knowledge obtained from these two cell culture systems with such different cellular organizations is complementary and, consequently, the study of both can give a more comprehensive view of tumor cell biology as a whole. However, the information that emerges must be compared. For this reason, high resolution proton nuclear magnetic resonance (1H-NMR) spectroscopy was used to determine if the same cell line (MG-63 human osteosarcoma cells) grown in monolayer or as small (about 50-80 microns in diameter), proliferating three-dimensional tumor spheroids with no hypoxic center has different structural and metabolic characteristics due to the spatial organization of the cells in each of the two cell models studied. In order to establish if these variations exist, the high resolution proton NMR (1H-NMR) spectra were obtained from both types of cultures and then compared. A new algorithm developed by our group for this purpose was utilized. The results seem to indicate that, in fact, the type of cellular spatial array determines specific changes in MG-63 cells. In particular, both energetic (as represented by alanine and lactate) as well as lipid (as represented by choline-containing metabolites and CH2 and CH3 groups) metabolism is different depending on the type of cell model utilized.

Posters

- Others -

19-5

Kinetic analysis of an enzyme reaction with inactivation in two steps and suicide substrate

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The inactivation of enzymes induced by a substrate takes place in enzymes acting on a substrate following a branched mechanism which consists of a catalytic route and an enzyme inactivation route. These substrates are called suicide substrates and they remain a very important and useful method in enzymology for studying enzyme mechanisms and designing potential drugs. There are enzymatic reactions that do not respond to the presence of a competitive inhibitor instantaneously. In some cases the inhibitor interacts slowly with the enzyme, in others the formation of the enzyme-inhibitor complex takes place in a very short time, and can evolve during a slow process of isomerization. In this contribution we present a kinetic analysis of enzyme catalyzed reactions evolving according to a Michaelis-Menten mechanism, in which an inactivation process in two steps takes place and simultaneously an enzyme inactivation is induced by an unstable suicide substrate. Under conditions of limiting concentration of enzyme and from the differential equations that describe the kinetics of the enzymatic system, we have obtained and proven the corresponding analytic expression for the concentration of the product of the reaction using numeric methods. We present a kinetic analysis based on the experimental progress curve of the product of the reaction. This work was partially supported by the Plan Nacional de Investigacion Cientifica (Project BQU2002-01960) and by Direccion General de Investigacion e Innovacion de la Consejeria de Ciencia y Tecnologia de la Junta de Comunidades de Castilla-La Mancha (Grupo Consolidado GC-02-032).

19-6

Fluctuations of active membranes : a study by contour analysis

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The physics of lipid membranes, first-developed-model system of biological membranes, has been widely studied at thermodynamical equilibrium. But, in a living cell, many channels and pumps dissipate energy in the membrane during ions transfer, and drive it in a non-equilibrium state. New fluctuation spectra of lipid membranes have been predicted by J. Prost and coll. (1-3) due to the non-equilibrium activity of proteins incorporated in membranes. It has been already shown (3-4) using micropipette experiments that the fluctuations of the membranes are amplified when a proton pump, the bacteriorhodopsin (BR), is photoactivated. In order to measure directly the fluctuation spectrum of active membranes (not measurable with micro-pipette experiments), we have developed new contour analysis experiments, which are not restricted to vesicles with a spherical shape. As a first step, we have improved the incorporation technique of the BR in giant liposome which can be transferred now to more fragile proteins and which allows an homogeneous distribution of proteins in the vesicles. The contour analysis has been first tested on passive membranes with different lipid compositions: the measured mechanical parameters are in very good agreement with published results. In presence of bacteriorhodopsin activity, the fluctuation spectrum is changed. The comparison with the theoretical model is currently in progress. References [1] J. Prost and coll., Europhys. Lett. 33(1996)321. [2] S. Ramaswamy and coll, Phys. Rev. Lett. 84(2000)3494. [3] J.-B. Manneville and coll, Phys. Rev. E 64 (2001) 021908. [4] J.-B. Manneville and coll, Phys. Rev. Lett. 82 (1999) 4356.

19-7

Towards the use of membrane protein/amphipol complexes in solution NMR studies

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'Amphipols' (APs) are amphipathic polymers that bind quasi-irreversibly to the transmembrane surface of membrane proteins and, thereby, keep them soluble in aqueous solutions in the absence of detergent (1,2). Using the transmembrane domain of the Escherichia coli protein OmpA (tOmpA) as a model system, we are examining the application of APs to NMR solution studies. The first HSQC spectra of tOmpA/AP complexes are encouraging, but they call for improvement. To this end, we are trying to understand the factors that determine the size, tumbling rate and monodispersity of tOmpA/AP complexes, and, therefore, the resolution of the spectra. Using size-exclusion chromatography, analytical ultracentrifugation, small angle radiation scattering, and binding measurements using radioactive or fluorescent APs, we are investigating the effects of the chemical structure of the AP, the ionic strength, the presence or absence of free APs in the solution, the temperature etc. A new method for the measurement of the diffusion coefficient of large particles by NMR has been devised (3). References 1. Tribet, C., Audebert, R., and Popot, J.-L. (1996) Proc. Natl. Acad. Sci. USA 93, 15047-15050 2. Popot, J.-L., et al. (2003) Cell. Mol. Life Sci., in the press 3. Ferrage, F., Zoonens, M., Warschawski, D. E., Popot, J.-L., and Bodenhausen, G. (2003) J. Am. Chem. Soc 125, 2541-2545

19-8

Cell adhesion molecules play an important role in the response of two osteosarcoma cell lines to 50 Hz sinusoidal magnetic fields

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Numerous epidemiological studies have reveled that extremely low frequency (ELF) sinusoidal 50-60 Hz magnetic fields of the strengths usually present in the environment due to the production and transport of electricity may have adverse effects on human health, particularly in promoting cancer. By contrast, many other reports have excluded this possibility. Cancer cell proliferation, death, invasion and metastasis are complex phenomena controlled by an even more complex series of pathways which communicate with each other through a myriad of signalling cascades. A pivotal role is played by cell adhesion molecules (CAM's) and their receptors. The CAM's principally involved are those directed against important components of the extracellular matrix such as fibronectin, collagen, laminin, hyaluronan, heparan sulfate and elastin. It was the aim of the present study to investigate the possibility that power frequency ELF fields can induce variations in the expression of CAM's in two human osteosarcoma cell lines (MG-63 and Saos-2). Specifically, the expression of two important integrins, VLA-2 (the receptor for collagen) and VLA-5 (the receptor for fibronectin), as well as CD44 were examined in both cell lines after their exposure for 7 and 14 days to a 50 Hz/0.5 mT field. Cell surface morphology, growth characteristics and death were also investigated. The results demonstrate that no variations in surface morphology and cell death were apparent between control and exposed cells in both MG-63 and Saos-2 cells while a few significant changes were noted in cell growth and CAM expression in treated cells.

Posters
- Others -

19-9

The effect of selenium on the accumulation of some metals in plants of Zea mays L treated with indole-3-acetic acid

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Selenium is a trace element, which has some important functions in living organisms in particular in animals and humans. Selenium is known to be essential to man and animals. Although for the animals its role is well known in the case of plants it still needs further investigations. In this study, we examined the relationship between the accumulation of selenite, the plant hormone (IAA-indole-3-acetic acid)and some nutrient elements (K, Na, Ca, Mn, Mg, Fe, Zn and Cu) in the tissues of roots, mesocotyls and leaves of Zea mays L. Our experiments were carried out with eight- and nine-day old maize plants (Zea mays L. var. K33xF2) grown on Hoagland's medium. Seeds of maize were cultivated in the darkness. Then, individual seedlings were transferred to an aerated solution containing the standard macro- and microelements, IAA and selenite, and cultivated in 12 hour light, 12 hour dark regime. The accumulation of the metals in the seedlings of maize was measured by emission spectroscopy using sequential spectrometer with excitation by argon inductively coupled plasma technique (ICP-AES). We observed that when selenite and phytohormone (IAA) are present in the external medium of growing plants, they change the uptake and accumulation of some cations in the leaf, mesocotyl and root tissues. The change of transport of some nitrient elements is probably one of the first observed symptoms of selenium's effects on plants.

19-10

Kinetic analysis of the steady-state and the transiennt phase of multicyclic enzyme cascades

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Enzyme cascades play an important role in the regulation of many physiological processes, e.g. regulation of metabolism, repair of lesions, protection against infectants and evolution. The special significance of the enzyme cascades is their ability to impose upper and lower boundaries on the rates of a biological process. Besides, the abundance of the design features in enzyme cascades provides many possibilities of response and adaptation to environmental cues and challenges. Such cascades are therefore essential to the success of evolutionary systems. The complicated structure of many enzyme cascades renders the kinetic analysis difficult. However, it is a prerequisite for the understanding of biological regulation at a high level.
In this communication presents a kinetic analysis of the whole course of the reaction, i.e. of both the transient phase and steady-state, of a class of multicyclic enzyme cascade systems. The kinetic equations for the important bicyclic cascades are obtained as particular cases of the general equations. Finally, equations for the fractional modifications are obtained which are valid for the whole course of the reaction. The steady-state expressions for the fractional modifications have been derived from the latter equations since they are not restricted to the condition of rapid equilibrium.
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19-11

Hofmeister effects on the colloidal stability of IgG-latex complexes

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Contrary to DLVO predictions, the stability patterns of colloidal particles are significantly influenced by the different type of electrolytes, having identical charge characteristics, used to coagulate the systems. In addition, the DLVO theory cannot either explain the increase in stability that is usually observed for many hydrophilic materials in presence of relatively high salts concentrations. This paper deals with this two non-DLVO phenomena. Actually, the present work is the second part of a broader study where polystyrene latex particles, protein molecules and various salts related to Hofmeister series (HS) are employed to test the colloidal stability and the electrophoretic mobility of different samples. In the first part of such a work (where no protein adsorption was performed), three hydrophobic latex of different charge characteristics were used in order to analyse the role played by both the surface charge sign and the surface charge density on ordering the selected Hofmeister ions. In the present paper, the same Hofmeister ions and a cationic latex of those previously used were chosen to study whether a change on the hydrophobicity of a surface, attained by means of adsorbing a protein (IgG), affects to the HS ranking given by the stability and electrokinetic patterns of the colloidal particles. The last part of the paper is focused on re-stabilization processes at high salt concentration paying special attention to the hydrophobic-hydrophilic character of the particle surfaces and the nature of the dissolved electrolytes. Most of experimental results are contrasted with theoretical electrophoretic mobility and stability data.

19-12

An in silico model for the optimization of threonine production in Escherichia Coli

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Threonine is an essential amino acid for birds and mammals and so there is considerable interest in its economic industrial production for a variety of uses. Its industrial production is insured by Escherichia coli overproducer strain. We have adapted and improved pre-existing dynamic model of central metabolism and threonine pathway by Chassagnole et al. (Biochem. J., 2001, 356,415-423 and Biotechnol. Bioeng., 2002, 79(1):53-73) to create a global in silico model of such a production strain. The aim of this work was to obtain strain improvements in the threonine production by DNA recombinant techniques directed to the controlling enzymes in the threonine synthesis. A study of the modification effect of the key branch points of the network (PEP and G6P node) provides us enough information to predict changes on threonine production. The model has been implemented with experimental results (enzyme activities, flux measurements and intracellular metabolite concentrations) from a bioreactor cultivation. The first part of the modeling procedure was the estimation of kinetic parameters (Michaelis and inhibition constants and rates maximum for each enzyme) by comparison between simulations and experiments. Then, we simulated the in vivo behavior of threonine production for identification of metabolic targets for genetic modifications. Finally, we checked the efficiency in threonine production of the optimized strains.

Posters
- Others -

19-13

Effects of flooding and cadmium poisoning on the membrane capacitance of pea seedlings

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Pea (Pisum sativum) seedlings germinated on vermiculite were transferred (after 3 and 10 days of growth) to half Hoagland hydroponic growth media containing 0, 200 and 400 micromole cadmium chloride. Other groups of seedlings grown on vermiculite were watered moderately with half Hoagland solutions containing 0, 200 and 400 micromole cadmium chloride. Electrical impedance spectra (800 Hz to 1 MHz) of shoots and roots of seedlings grown in hydroponic solutions and in vermiculite were measured daily during 5 days of growth (beyond the initial 3 or 10 days of germination). Measured impedance spectra were approximated by modified Hayden models, and apoplasmic and symplasmic resistances and membrane capacitances were estimated. Membrane capacitances of both shoots and roots were lower for flooded seedlings (grown hydroponically) than for seedlings on vermiculite. These decreases in capacitance can be explained by increased water content in apoplasm and symplasm, which is proven by decreased apoplasmic and symplasmic resistances for seedlings in hydroponic growth media (relative to seedlings in vermiculite). Cadmium in hydroponic growth media and watering solutions increased membrane capacitances for roots of seedlings germinated for 3 and 10 days. The effect of cadmium on the membrane capacitance in shoots was observed only for seedlings germinated for 10 days. The reduction of water uptake caused by cadmium poisoning (as previously reported in the literature) can explain the observed effects.

19-14

Proteins overexpression induced by cadmium in Posidonia oceanica.

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Marine phanerogams are an interesting model system to study molecular mechanisms involved in the adaptation to environmental stresses, because basic understanding of their physiology is still limited. Furthermore, it is known that seagrasses, in particular Posidonia oceanica, may absorb and accumulate metals from sediments in its organs and tissues (Maserti et al., 1988; Rainbow and Phillips, 1993), determining metal bioavailability in the marine ecosystem. Trace metals accumulation in terrestrial plants may induce the synthesis of phytochelatins and /or metallothioneins, a low molecular weight proteins, whose role in the plants detoxification to trace metals is still controversial (Zenk, 1996). In a previous study (Giordani et al. 2000), three DNA sequences putatively encoding MTs were isolated by PCR and an accumulation of MT RNAs were observed in Posidonia plants experienced with cadmium. Here, a study where mono and bidimensional electrophoresis were used to identify overexpressed proteins in Posidonia plants exposed at two concentrations of cadmium (10 uM and 100uM) is reported. From our results, the metal seems to induce the over expression of proteins with a molecular weight in the range of 10-14kD, that could belong to the metallothionein family. The overexpression effect seems dependent on the metal concentration. The final aim of our research will be the characterization of the aminoacidic sequence of these proteins. B.E. Maserti, R. Ferrara, P. Paterno (1988). Mar. Poll. Bull. 19: 381-382. P.S. Rainbow, D.J.H. Phillips (1993). Mar Poll. Bull. 26 (593-601). M.H. Zenk (1996). Gene 179: 21-30 T. Giordani, L. Natali, B.E. Maserti, S. Taddei, A. Cavallini (2000). Plant Physiol. 30: 1571-1581.

19-15

Scattering techniques to help crystallization

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The explosion of data coming from genome sequencing provides us with numerous proteins to crystallize. The development of high throughput platforms for crystallization and the improvement of X-ray sources, data collection instruments and data analysis facilitate the rapid determination of 3D structures of biological macromolecules. But, despite this development of technology, only 10 to 50% of crystallization trials succeed in growing good quality crystals. This limitation is mainly due to the fact that crystallization is not yet a well controlled process with general rules. For some years, we have developed physical approaches (small angle X ray scattering, and light scattering) to study interactions between biological macromolecules in solution as a function of crystallizing agents. We have characterized effects of different physicochemical parameters on interactions between proteins, which are favourable to their crystallization. Different biological systems with different sizes, molecular weights, charges (pI) were studied as a function of pH, temperature, concentration and nature of salt, percentage and size of polymer. With the use of the second virial coefficient, which permits to quantify interactions between particles in solution, we can choose crystallizing agents able to induce attractive interactions (i.e. with negative second virial coefficient). Thus we have shown with large proteins like Urate oxydase and Apoferritin, that salt is not effective to induce attractive interactions and that polyethylene glycol can induce attractive interactions when the electrostatic repulsion is low (when pH is close to pI or with some salt far from pI).

19-16

Structural analysis of altered human chromosomes by atomic force microscopy

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Structural abnormalities of human metaphase chromosomes, fixed according to standard procedures for optical microscopy but not stained, were detected by atomic force microscopy (AFM). In fact, chromosomes obtained from Roberts syndrome subject, as well as rearranged chromosomes - that is chromosomes with gaps, breaks and exchanges - were imaged by AFM. In particular, Roberts syndrome chromosomes represent an enigma for their morphology: they are separated at centromeric level, sister chromatids have a road track appearance and no information is yet available for their structure. Our analysis show that Roberts syndrome chromosomes retain their morphology with clear p and q arms, however their chromatid are fully separated and no structure were imaged between them. Regarding rearranged chromosomes, our analysis provided useful information to distinguish chromatid gaps and breaks and provide a possible explanation for their formation. These observations indicated that AFM is a useful tool for analysis of chromosome aberrations associated with human pathology.

Posters

- Others -

19-17

Molecular recognition studies on cyclodextrin-based glycoclusters and glycodendrimers

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We are involved in the construction of site-specific drug delivery systems based on cyclodextrin (CDs)-glycocluster conjugates having dual function as host for the complexation of guest molecules and lectin ligands. [1] In addition to show strong lectin binding affinity due to the so-called cluster effect, we have shown that the chemically modified CDs are able to form inclusion complexes with several guest molecules. We found that the inclusion complexation ability of 14-mer glycoCDs with some chosen guests is very small or in some cases apparently non-existent. Therefore, we became interested in exploring the type of compounds that would form inclusion complexes with those glycoCDs. We chose glycoCDs containing seven and fourteen carbohydrate units (rhamnose and lactose) and we used the amphypatic molecule DAUDA and adamantanecarboxylate (AC), with a quasispheric symmetry, as model guest molecules. Isothermal titration calorimetry, fluorescence spectroscopy and RMN have been used to investigate the stoichiometry and thermodynamic parameters of these complexes with the glycoCDs. In order to obtain the heat capacity change, ΔC_p^o for these complexes, the binding has been performed at three temperatures in the range of 18 to 30ºC. The binding reactions are enthalpically driven with little change in the heat capacity on binding, and exhibit enthalpy-entropy compensation. The thermodynamic parameters are rationalized in terms of the possible interactions between those guests and the features of the glycoCDs.

[1] a) Ortega-Caballero et al (2001) J. Org. Chem. 66, 7786-7795; b) Vargas-Berenguel et al (2002) Chem. Eur. J. 8, 812-827.

19-18

The Editorial Process for The Physical Review.

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I will give an overview of the editorial process for The Physical Review, addressing questions frequently asked by authors and others in the scientific community. The description of the review process will address questions such as how reviewers are chosen, why the process is not quicker, and what authors can do to help make the process quicker and smoother. I will also address other comments and concerns about the journal and about the Biological Physics Section in Physical Review E, in particular.