Posters

– Morphogenesis: from cell adhesion to organs –

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Mimicking cadherin-mediated cell-cell adhesion

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Cell adhesion is considered to play an important role in many biological functions such as the regulation of organ and tissue development during embryogenesis, and the maintenance of normal tissue structure in the adult organism. The specificity of cell-adhesion is controlled by genetic expression of receptors at the cell surface, called cell adhesion molecules (CAMs). One of the major families of CAMs is the cadherins that initiate intercellular junctions by homophilic ligation of their extracellular domains in the presence of calcium. The aim of this work is the creation of a biomimetic system, which permits to understand mechanisms by which a defined number of cadherin molecules coordinate cell-cell adhesion with dynamic changes in the cytoskeleton. To address these questions, we have developed patterned surfaces, based on the block copolymer micelle nanolithography, where geometrical well-defined gold dots are functionalized by a chimera of the cadherin ectodomains fused to the Fc region of human IgG1. Using an AFM, we measure the strength of the interactions between the cadherins onto the nanodots and the cadherins at the cell surface. First, to vary the cadherin distance allows us to apply nanopatterned substrates as a kind of "nanoruler" to measure important length scales in cadherin molecules. Second, we control the number of binding sites offered to the cell and this allows us to probe potential cooperative effects in cell adhesion phenomena through controlled cadherin clustering by clear chemical and physical means.

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Role of glycocalyx in cell adhesion studied by colloidal probe techniques on biomimetic systems

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Combination of reflection interference contrast microscopy and laminar flow chamber technique is used to probe the functional roles of the glycoprotein coat of cells. A model of glycocalyx is reconstituted by grafting long polymers on glass while codeposition of adhesion molecules induce specific binding of functionalized microbeads. The transient adhesion of the colloidal probes followed in three dimensions provide accurate information on ligand-receptor binding kinetics and its modulation by the surrounding polymeric layer.

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Measuring the adhesion and mechanical properties of individual cells using atomic force microscopy

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For the last decade people have been using the AFM to measure colloidal interactions. This is achieved by attaching a particle onto an AFM cantilever and measuring that particles interaction, either with a second particle, or more usually a flat surface made of a similar material to that of particle. Initially DLVO forces were measured, quickly followed by steric and hydrophobic interactions. The AFM has been used also in the field of biology, largely as an imaging technique, but also to measure the strength of specific interactions such as antibody-antigen interactions.
In this presentation I shall describe a method whereby single living biological cells, namely yeast, erythrocytes and fibroblasts can be attached to AFM cantilevers, and their interactions with various surfaces have been determined. The technique enables the adhesion of cells to surfaces to be studies and results for a range of surfaces will be presented. One problem encountered during this work, which has proven to yield interesting mechanical details about the cells was that during an experiment the cells deform. By comparing data of the interactions of the cells with a surface and the bare cantilever with the surface, it is possible to estimate the deformation of the cell. It is found that the deformation is well described by Hertz theory of elasticity, enabling us to estimate the Young's modulus of the cells: yeast 0.65+/- 0.15 MPa; erythrocytes 0.25+/-0.1 MPa; and fibroblasts 20+/-10 kPa.

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The use of impedance spectroscopy to monitor barrier integrity and cellular differentiation

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Electrical impedance spectroscopy was used to monitor the development of a maturing differentiated state of mono-layers of cells during culture. The cells utilised in this study were the endothelial-like cell line ECV304 and the epithelial colonic cell line Caco-2. The cells were grown on a permeable support membrane in mono-culture and following co-culture with the astrocytic cell line 1321NI. Electrical impedance measurements were made applying a transversal alternating current through the culture using four pairs of internal electrodes over a frequency range (1300-1900 kHz). Measurements of Trans-cellular Electrical Resistance were used to monitor the presence of tight junction's barrier integrity. The data present here permit the assessment of the ability of electrical impedance spectroscopy to monitor the formation of tight junction integrity as an indicator of cellular differentiation. Results show a good correlation of impedance measurements with culture growth and indicate sensitivity to the detection of the increasing tight junction integrity/complexity and offer a non-destructive method for monitoring differentiation in barrier culture systems.

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Bioreactor for cultivation of human mesenchymal stem cells on porous matrices

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Mesenchymal stem cells (MSC) are anchorage-dependent adult cells capable of autoreplication and differentiation towards the cell types from their tissue of origin. Potential applications in cell therapies and tissue engineering have elicited vivid interest for their cultivation and characterization. Aiming to optimize the culture conditions of MSC seeded on biocompatible scaffolds, we built an oscillating perfused column (OPC) bioreactor. It ensures sterility, temperature control, gas exchange and laminar flow of the cell culture medium in the vicinity of the tissue constructs. Our OPC system relies on a modular design, incorporating 1 to 5 cylindrical tissue culture flasks in a transparent support box whose temperature is maintained at 37 °C. A peristaltic pump circulates the medium along the tygon tubing that connects the flasks in parallel and a gas exchanger in series with them. The latter contains gas permeable silicone tubing for equilibrating the medium with an atmosphere augmented with 5% CO_2, eliminating the need for an incubator. The perfused columns are maintained in vertical position until construct sedimentation, then the support is mildly rotated by 180 degrees, thereby keeping the tissue constructs in a continuous fall within the medium.

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Adhesion-driven morphogenesis in living tissues studied by computer simulations

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Self-assembly is a process whereby interacting entities spontaneously form ordered structures. It hinges on the mobility of the components and on the reversibility of their associations. Biological morphogenesis may be viewed as the self-assembly of living cells into functional tissues. To explain it, Steinberg proposed the differential adhesion hypothesis (DAH), which states that cells possess a type-dependent adhesion apparatus and are motile enough to reach the lowest energy configuration. We developed a DAH-inspired lattice model of living tissues in interaction with their environment, and performed Monte Carlo simulations of the emergence of specific, biologically relevant shapes. The model was tested against experiments involving CHO cells, and generalized for several types of cells and embedding media. We simulated cell sorting versus interactions with the surrounding medium, tube formation from rings of aggregates closely packed along their symmetry axis, lumen formation by contiguously placed spheroids made of gel cores wrapped in cells, and the development of an endothelialized tube when the superimposed rings were made of two types of cells and the medium in the interior of the tube was different from the external one. We incorporated cell proliferation and studied the energetic conditions for epithelial-to-mesenchymal transformations, which play a central role in embryonic heart development.

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Human mesenchymal stem cells for bone and cartilage tissue engineering: isolation and culture

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Stem cells are immature cells with a remarkable viability and proliferative capacity, able to generate various mature cell types. Mesenchymal stem cells (MSC), can give rise to several cell types, including osteoblasts, adipocytes, chondrocytes. We studied human MSC as cell sources for tissue engineering. Using bone marrow extracted from 9 patients, MSC have been isolated by three different methods: (1) the Ficoll-Paque technique for the isolation of mononucleated cells followed by the separation of MSC by adherence to plastic, (2) Ficoll-Paque followed by immuno-magnetic separation of MSC using a CliniMACS system and (3) a negative selection of MSC by the RosetteSep technique followed by adherence to plastic. Since the composition of the cell culture medium is a decisive factor in preserving stem cell features, we optimized our media, assuring a good reproducibility of the results. On the 3rd day after seeding the isolated mononucleated fraction, we observed elongated adherent cells which have undergone extensive proliferation between days 5 to 9, with colony formation around days 10 to 14. The cell population reached 70–80% confluence within 3 weeks. Independent on the isolation procedure, the largest number of cell colonies was obtained for a seeding density of 10^6 cells/cm^2. The cells have been characterized immunophenotypically by flow cytometry and the expression of specific molecular markers was revealed by RT-PCR.

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Cell movements and mechanical forces during the migration of Dictyostelium slugs

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Dictyostelium provides an experimentally accessible and simple model system to investigate many biological processes. It is assumed that chemotactic waves organize the periodic motion of cells during the migration of *Dictyostelium* slugs. However, the mechanisms by which mechanical forces are exerted, their magnitude and their location, are unknown.
We present here the first measurements of the distribution of forces exerted by slugs using the elastic substrate method. Deformation field is measured with a confocal microscope from the displacement of fluorescent beads embedded in soft elastomer substrates. We show that force calculations are simple and robust when the noise level on bead displacements is low. We are able to identify clearly separate friction areas in the tip and in the trail, and traction in the prespore area [Rieu. *J. Biol. Phys.,* 30(2004)345]. Surprisingly, the magnitude of friction and traction forces is decreasing with slug velocity indicating that these quantities are probably related to the dynamics of cell/substrate adhesion. Contrary to what is assumed in models and simulations, friction is not a viscous drag but rather close to solid friction. We also measure large perpendicular forces around slug boundary suggesting an large role of the sheath in the transmission of forces to the substrate. We will present simple scaling arguments explaining how this complex force pattern may generate a well defined length-dependence of slug velocity.

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Force mapping in epithelial cell migration

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We dynamically measure the traction forces exerted by epithelial cells on a substrate. Most of the previous on stresses at the cell-to-substrate interface uses deformations within elastic materials such as thin polymer films or thick polymer gels. We have developed an alternative approach based on a force sensor made with a high density array of elastomeric microfabricated pillars under the cells. Traction forces induced by cell migration are deduced from the measurement of the bending of the pillars. This technique, compatible with fluorescence microscopy, uses a multiple particle tracking method to estimate the mechanical activity of cells in real time with a high spatial resolution ($\sim$2 μm) imposed by the periodicity of the posts array. For these experiments, we use differentiated and polarized Madin-Darby canine kidney (MDCK) epithelial cells. The maximum intensity of the forces is localized on the edge of the epithelia. Hepatocyte growth factor (HGF) induces a migratory phenotype in MDCK cells. We compare forces generated by MDCK cells *versus* HGF treated MDCK cells and correlate traction forces with actin distribution and the expression of focal adhesion proteins.

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Effect of substrate rigidity on forces applied by cells by using an array of flexible micropillars

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We have developed a substrate made of a high density array of elastomeric micropillars to measure traction forces exerted by cells on their substrate. Previous studies have shown that cell movement and focal adhesions are regulated by physical interactions at the cell-substrate interface. We have shown that traction forces and focal adhesions are strongly correlated to the rigidity of the substrate by analyzing the force *versus* rigidity depending relationship. Indeed, we culture MDCK epithelial cells on substrates of elastomeric microfabricated pillars coated with fibronectin. Using a multiple particle tracking home-made software, we measure the traction forces by analyzing the local deformations of the pillars. We vary the spring constant of the micropillars by changing their geometrical parameters, length and radius, to obtain a broad range of rigidities from 2nN/μm to 130nN/μm. Forces exerted by cells on their substrate linearly increase with the rigidity of the pillars. These observed changes are correlated with the expression of focal adhesion proteins. An increase of vinculin expression is observed on stiffer substrates.

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Adsorption and adhesion of giant liposomes monitored by electrochemical impedance spectroscopy

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Adsorption and adhesion of giant unilamellar vesicles containing different concentrations of biotinylated lipid on avidin were monitored quantitatively and in real time by electrochemical impedance spectroscopy. This method is based on measuring changes in AC impedance of small gold-film electrodes. At a frequency of 40 kHz the surface coverage is directly proportional to the changes in capacitance. This technique also allows distinguishing between intact and disrupted liposomes. The time courses of these changes were simulated by using a random sequential adsorption (RSA) model. Additionally, the equilibrium coverage fluctuations were measured. By using Fourier transformation of the fluctuations it is possible to extract the rate constants governing the adsorption kinetics. Comparison with adsorption of latex beads shows that the adsorption kinetics is mainly controlled by the interaction of biotin and avidin and that deformation of the vesicles determines the large response.

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Modulation of cellular adhesion by chemical cross-linking of polyelectrolyte multilayer films

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Polyelectrolyte multilayers (PEM) are a new attractive way to create biofunctionalized surface coatings. Cell adhesion can be modulated by changing the physical properties of the film by cross-linking. The aim of this work was to investigate primary cells response towards film stiffness by chemical cross-linking. For this purpose, chondrocytes primary cells were chosen. Cross-linking was achieved by means of a simple water based protocol using EDC in combination with sulfo-NHS. After six days of culture on native and cross-linked films, MTT assays show that cross-linked films are ten fold more favorable to chondrocytes adhesion and proliferation than native films. The images obtained by CLSM show a deformation in the cell contact zone of native films. The films may be too soft for the cells to create strong anchors. In contrary, on cross-linked films, chondrocytes can anchor in the film and no deformation of the film is visible. AFM images on cross-linked films show widely spread primary chondrocytes with typical polygonal shapes. The change from a non adherent to an adhesive surface after cross-linking may originate from the increased rigidity of the cross-linked films (about 250 kPa compared to 30 kPa for the native ones, as measured by AFM colloidal probe). Such effect of matrix stiffness on cell behaviour has always been investigated for relative thick gels, but was never investigated for thin films. The polyelectrolyte multilayers represent a new way to address this question.

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Neutrophil spreading: from touchdown to first steps

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Time resolved dynamics of spreading of human neutrophils following activation was observed by Reflection Interference Contrast Microscopy (RICM). Images were analysed to identify simultaneously the changes in the overall cell shape and the zones of close contact with the substrate and these were followed over time. We show that in case of neutrophils, cell spreading is anisotropic and directional from the very beginning resulting in a translation of the cell centre even as the cell spreads; in other words, the cell undergoes locomotion as it spreads. The curve describing the spreading area of the cell as a function of time can be fitted piecewise as a series of power law functions. All cells exhibit an initial slow spread regime followed by a fast spread regime, each characterised by a different exponent of the power law function. The different spreading regimes are related to changes in the adhesion state and/or dynamical state of the cell. The instantaneous velocities of cell membrane segments are calculated. Spreading is found to be a result not of monotonous and directional movement of the membrane but a series of apparently random fluctuations.
Cells are similarly followed when they complete their spreading phase and start to migrate. RICM data is correlated to traction forces exerted by the cells.

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The Micro Flow Chamber Chip Cell Adhesion on a Planar Surface

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The adhesion of cells plays a crucial role in a variety of biological processes. Classical parallel-flow-chamber experiments showed that understanding adhesion means to understand the hydrodynamic forces acting on the cell. However, these forces vary enormously with the geometry of the system..In order to understand the effect of small (10 – 20μm) arteries, curved and branched vessels on the adhesion mechanism these geometries need to be mimicked. Therefore an adaptable set up needs to be designed.
However, the parallel-flow chamber is restricted to one defined flow channel geometry, has a dead volume of several millilitres and contains multiple moving parts which can easily become clogged or contaminated. The "micro flow chamber" on a chip can easily be adjusted to any geometry using "soft lithography", contains no moveable parts, and carries a volume of 100 – 1000 times less then conventionally used flow-chambers, allowing for the performance of experiments with most valuable substance (e.g. monoclonal AB). Due to the chips optical transparency the entire system can be placed under the microscope enabling us to observe the cells online.
With this setup we were able to study the effect of branched vessels on the adhesion of melanoma cells for the first time.

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Biophysical characterization of the cell-substrate contact

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Microelectronic-based biosensors that allow non-invasive recordings of cellular activity are in focus of current developments. This technique is based either on microelectrode arrays or open insulated field-effect transistors and holds great promise for biomedical studies. However, the mechanisms underlying the cell-substrate coupling are not completely understood. We use different approaches to characterize the electronic contact between the substrate surface and the attached cells in detail.
The small cleft between the cell membrane and sensor surface was topographically analyzed by means of TEM of fixated HEK293 cells, which have been cultured on different substrates: We found a non-homogenous distribution for the cleft distance and could clearly distinguish between focal adhesion regions of the cells and areas with an increased distance of the cell membrane. The mean distance was in the range of 100 nm.
The cleft was also electrically characterized by analyzing the transfer function of an applied ac-voltage: We observed significant differences in the transfer functions of the sensors, when a cell is closely attached to the sensor spot of the devices.
The observed differences in the cleft distance and the transfer functions coincide with the recorded extracellular signal amplitudes. Further work will focus on combining these results to develop an enhanced model of the cell-substrate coupling.

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The behavior of MC3T3-E1 cells on superficially modified poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)

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The cells adhesion, spread, growth, proliferation and migration on biomaterials have been widely studied for its importance in biomedical materials and tissue Engineering. In this article, MC3T3-E1 cell adhesion and proliferation on poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx), a new member of polyhydroxyalkanoate(PHA) family, was investigated with physical, chemical and biological methods. The results showed that the response of cells to original and surface-hydrolyzed PHBHHx films was different: MC3T3-E1 cell adhesion and proliferation on surface-hydrolyzed PHBHHx was significantly greater than that on original PHBHHx films, which was contributed to presenting carboxyl and hydroxyl groups to the surface of the material and modification of surface morphology of the material with surface hydrolysis. Therefore, surface hydrolysis makes PHBHHx become a more suitable biomaterial for MC3T3-E1 cell response and for application in bone tissue engineering.

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Nonaxisymmetric phospholipid vesicles: rackets, boomerangs, and starfish

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Within the area difference elasticity model, we numerically minimize the bending energy of phospholipid vesicles to study the nonaxisymmetric shapes at the oblate-prolate transition. We analyze the onset of a coherent structural hierarchy of hybrid shapes such as rackets, boomerangs, and starfish, which all consist of a flattened body and one or more elongated arms. The phase diagram of these shapes is characterized by a critical point terminating the line of discontinuous transitions between rackets and boomerangs/starfish. The critical point is located very close to the discocyte with the volume of a human erythrocyte, implying that in this part of the parameter space minute variations of volume and area difference can induce large changes of shape.

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– Exhibitors Symposium –

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Characterization of protein hydration: a new approach to crystallization and folding problems

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The vital biological functions of proteins in the cell are based on their intact native structure. Stabilized by water, the structure of proteins is highly dependent on the equilibrium with its environment (hydration state) which can be significantly influenced by external factors such as temperature, ionic strength or drugs. Disturbance of this sensitive equilibrium often leads to misfolding and aggregation. The recent development of molecular acoustics opened up new application possibilities including high resolution observation of hydration changes in proteins. Characterization and monitoring of protein hydration has been used as an efficient approach for the structural and thermodynamic analysis of proteins. Thereby, molecular acoustics assisted the finding of optimal crystallization conditions.

Here we demonstrate the sensitivity of molecular acoustics by monitoring protein folding and stability. Thermal denaturation and renaturation of several proteins were characterized using temperature scans and revealed significant differences in general folding behaviour, the number of folding intermediates, general thermal stability and renaturation efficiency. Thus, molecular acoustics proved to be an efficient analytical approach to characterize the structural behaviour and stability of various proteins under native conditions.

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Combining advanced force and optical microscopy techniques for biophysics research

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The range of biophysical applications for atomic force microscopy (AFM) continues to grow, as the advantages of high resolution imaging in physiological environments are combined with measuring and manipulating the structures under investigation. A key factor is the ability to combine AFM with advanced optical techniques, such as phase contrast, DIC and epifluorescent, TIRF or confocal imaging. This enables correlation of the high resolution structural information with specific labelling of active molecules. The BioCell allows full environmental control of samples on coverslips in liquid, for maximal optical resolution, single molecule imaging and spectroscopy, and cell studies.

The CellHesion development kit has been specially designed to extend the capabilities of AFM in cell binding and recognition assays, giving reproducible and quantitative analysis of cell-cell and cell-substrate binding forces. Single cells within a culture can be selected, attached to a flexible cantilever and subsequently allowed to adhere to a second, specific cell or region of substrate. Simultaneous information from advanced optical techniques gives insight into many additional cellular processes that occur on binding, such as changes to actin structure, calcium flushes, distribution of labelled proteins, or morphological changes.

[1] Poole et al. FEBS Letters, 565: 53-58 (2004)
[2] Poole and Müller British J. Cancer 92:1499-1505 (2005)

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– Functional Complexes –

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G-lego: a novel entry of ultra-stable higher ordered aggregates generated by self-assembling of GROs

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Structures of short ssDNA molecules that are purine rich and contain runs of guanine-rich tracts are currently of strong interest since they are known to relate to various aspects of biological functions including telomers, antiproliferative phenomenon and others. Another aspect which evokes strong interest is the tendency for self-aggregation resulting in higher ordered structures or aggregates in a large scale based on their potential to form G-quartets. However, the mechanism involved in this phenomenon has been left unsolved. In this context, here we investigated the molecular interactions and self-assembling behavior of a series of simple guanine-rich oligonucleotides (GROs) by electrophoresis, CD and AFM methods. A novel GROs, $d(G_{11}X)$, where X is non-G base, were found to generate ultra-stable aggregates bearable against the denaturants and nucleases. The CD spectra was supportive for the notion that $d(G_{11}X)$ is forming a mixture of parallel and anti-parallel quadruplex structures. AFM observation of $d(G_{11}X)$ clearly showed the image of aggregates (average height of 3 nm, i.e., approximately four times of the measured height of dsDNA) and making them distinct from the aggregates of other highly similar GROs. Based on the facts thus revealed, we constructed a model, 'G-lego', for the aggregation phenomenon: successively attaching of a unitary G-quartet block through a mechanical switching of Hoogsteen-type pairing like a modular toy 'lego' to explain this for further investigation.

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Modelling and mutagenesis studies of a substrate-specific enhancer of tolloid metalloproteinases

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Procollagen C-proteinase enhancers (PCPEs) are extracellular matrix glycoproteins that can stimulate the activities of tolloid metalloproteinases by up to 20-fold. Tolloid metalloproteinases process several extracellular substrates, involved in matrix assembly, cross-linking, cell migration and growth factor signalling. PCPEs consist of two CUB domains (required for enhancing activity) followed by an NTR domain. We have recently shown that PCPE-1 specifically enhances C-terminal processing of fibrillar procollagens, with no effect on other tolloid proteinase substrates. To identify the structural features of PCPE-1 involved in enhancing activity, we modelled the CUB1 domain, based on the known 3D structures of other CUB-containing proteins, MASP-2 and its alternatively spliced form MAp19 (3), both of which bind to the collagen-like region of mannan binding lectin (MBL). Residues involved in the binding of MAp19 to MBL are located in surface loops close to a calcium binding site. Three such residues are highly conserved in CUB domains of various origins, including TYR57 in PCPE-1 CUB1. Three other residues (including GLU16 and THR79 in PCPE-1) are specific to the CUB1 domains of PCPEs. Mutation of TYR57 in PCPE-1 CUB1 to alanine led to a reduction in enhancing activity of approximately 80 %. In contrast, alanine mutations of GLU16 and THR79 had little effect on enhancing activity. This work sheds light on the biological roles of CUB domains found in numerous extracellular and plasma membrane-associated proteins.

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The megaDalton Cellulosome from Clostridium thermocellum: building the puzzle

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The *Clostridium thermocellum* is a bacterium from hot springs and, similarly to other bacteria, it converts hemicellulose into ethanol. These microorganisms express multienzyme complexes dedicated to the degradation of the plant cell wall. These complexes, Cellulosomes, are composed of modules assembled by an integrating protein, the scaffoldin, which is made of several type I cohesins that bind the type I dockerins of the enzymatic modules. A type II dockerin of the scaffoldin binds to a type II cohesin and anchors the whole complex to the cell surface. Other modules, named Carbohydrate Binding Modules (CBM), are responsible for adherence to the substrate. The type I cohesin-dockerin complex structure has been solved and it shows that the β-sheet domain of the cohesin interacts with one of the helices of the dockerin. Sequence duplication in the dockerin results in internal 2-fold symmetry, suggesting that both "halves" of the dockerin may interact with cohesins. The 2.5Å structure of the type II cohesin has also been solved. Subtle differences between type I and type II cohesins explain why these modules display distinct specificities for the target dockerins. We also report the 1.98Å structure of the family 11 CBM belonging to a cellulosomal enzyme. The structure of the CBM11 reveals a concave side that forms a potential carbohydrate binding cleft.

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Citrate-dependent and heparan sulfate-mediated cell surface retention of cobra cadiotoxin A3

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Anionic citrate is a major component of venom, but the role of venom citrate in toxicity is poorly understood other than its inhibitory effect on the cation-dependent action of venom toxins. By immobilizing Chinese hamster ovary (CHO) cells in microcapillary tubes and heparin on sensor chips, we demonstrated that heparan sulfate (HS)-mediated cell retention of the major cardiotoxin (CTX) from the Taiwan cobra, CTX A3, near membrane surfaces is citrate dependent. X-ray determination of a CTX A3-heparin hexasaccharide complex structure at resolution 2.4 Å revealed a molecular mechanism for toxin retention in which heparin induced conformational changes of CTX A3 lead to citrate-mediated dimerization. A citrate ion bound to Lys23 and Lys31 near the tip of loop II stabilizes hydrophobic contact of the CTX A3 homodimer at the functionally important loop I and loop II regions. Additionally, the heparin hexasaccharide interacts with five CTX A3 molecules in the crystal structure, providing another mechanism whereby the toxin establishes a complex network of interactions that result in a strong interaction with cell surfaces presenting heparin. Our results suggest a novel role for venom citrate in biological activity and reveal a structural model that explains cell retention of cobra CTX A3 through HS-CTX interaction.

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– Functional Complexes –

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Characterization of the interaction of PTB1 protein with RNAs

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Polypyrimidine Tract Binding protein (PTB) has four RNA Recognition Motif domains (RRM). PTB1 protein binds to polypyrimidine tracts of pre-mRNA introns and was shown to be involved in regulation of splicing. PTB1 has been shown to interact with the Hepatitis C Virus (HCV) 3' Non-Translated Region (NTR). The 3'NTR has a variable region, a Polypyrimidine Tract and a highly conserved 98 nucleotides sequence (X RNA).

We propose to characterize PTB1 protein interaction with two RNAs: the HCV 3'NTR and $GABA_A\gamma2$ pre-mRNA. Gel mobility shift experiments show that several PTB1 binding events occur cooperatively for both RNA targets. Stoichiometry shows that six and eight PTB1 proteins bind to HCV 3'NTR and $GABA_A\gamma2$ pre-mRNA, respectively. These experiments also demonstrate that PTB1 RRM1/2 prefers to bind to the sequence U/C whereas PTB1 RRM3/4 binds to U/U. Additional investigations reveal two distinct modes of interaction of PTB1 with its two RNAs. Two PTB1 or two PTB1 RRM1/2 proteins bind in a non-cooperative manner to the X RNA with the same affinity (K_D = 800 nM). PTB1 RRM3/4 does not bind to X RNA. On the other hand, PTB1 binds cooperatively with high affinity (K_D = 10 nM) to $GABA_A\gamma2$ pre-mRNA. PTB1 RRM3/4 has a lower affinity than PTB1 suggesting that the domains RRM1 and 2 are contributing to the tighter interaction of PTB1. Toe-printing experiments map PTB1 binding sites on these two RNAs. We will present models for the interaction of PTB1 with RNA.

P-545

Structural study of bacterial glutamate synthase (NADPH-GltS) by 3D cryoelectron microscopy and SAXS

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Glutamate synthases are a class of complex iron-sulfur flavoproteins, subjected to a sophisticated control mechanism through protein-ligand and protein-protein interactions. Both the bacterial type NADPH-dependent glutamate synthase (NADPH-GltS) and the plant-type ferredoxin-dependent enzyme form (Fd-GltS) are biochemically and functionally well characterized, but structural information is limited to the crystallographic structures of the isolated alpha subunit of NADPH-GltS and of the homologous single polypeptide chain of Fd-GltS.
We have calculated a 3D cryoelectron microscope map of NADPH-GltS, allowing us to identify the stoichiometry and the relative positions of alpha subunits and molecular models of beta subunits. By combining SAXS and 3D cryo-EM, we hope to improve the resolution of our 3D map.

P-546

Proton NMR detection of porphyrin derivatives in small unilamellar vesicles

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Small Unilamellar Vesicles (SUV) of phospholipids are known to permit proton and phosphorous Nuclear Magnetic Resonance of the lipids through a relatively fast tumbling. However, the extent of the proton NMR detection of molecules associated with the phospholipids is not well documented. In the case of the porphyrin derivatives, we will show the drastic influence of the molecule/phospholipid ratio in the linewidths of the proton resonances of the macrocycles. These results have been obtained with the paramagnetic hemin complexes and the diamagnetic hematoporphyrin. Preliminary results involving the detection of the methyl resonances of cytochrome *c* interacting with SUV will be also presented.

P-547

Analytical ultracentrifugation and membrane proteins

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Analytical Ultracentrifugation (AUC) is a unique and important technique for the characterization of macromolecular assembly and interactions in solution, because it is firmly based on rigorous hydrodynamic and thermodynamic theory. AUC provides the hydrodynamic radius and buoyancy molar mass (related to the composition of the particle, including protein-solvent interactions) of a homogeneous ideal solute. For interacting systems, it provides values for the association constants and/or the second virial coefficients (related to weak inter-particle potentials).
For the study of membrane proteins, protocols using different experiments can be used -in conjunction with the most recent data treatment- to describe the behavior of the amphipatic compounds in solution, homogeneity of the membrane protein solutions, amount of bound detergent in the complex, oligomeric states of the protein and protein-protein association constants. Different approaches and results will be described. The success of these approaches could allow studying the link between protein-detergent and protein-protein interactions.

Posters

– Functional Complexes –

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Physicochemical basis of the regulation of a M. tuberculosis FHA-domain protein by phosphorylation

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The sequencing of the genome of the pathogen *Mycobacterium tuberculosis* has revealed many unexpected "eukaryote-like" features, amongst which the presence of seven proteins containing Fork-Head Associated (FHA) domains. The presence of an 11-stranded β sandwich FHA domain in a protein is considered as an indication that its interactions are regulated by reversible phosphorylation on threonines of its partners.
Here, we focus on protein GarA (Rv1827), of yet unknown function. GarA contains an autonomous folding FHA domain in its C-terminus, whose boundaries were determined by limited proteolysis and bio-informatic methods.
Interestingly, we show that GarA is itself a substrate of several *M. tuberculosis* Ser/Thr kinases (STPKs), and that it is specifically phosphorylated on a single threonine in its N-terminal extension. The impact of this phosphorylation event on the conformation and stability of GarA was determined by circular dichroism and differential scanning calorimetry.
Furthermore, we investigated the mechanism of recruitment of GarA by the STPKs, and characterized the physico-chemical properties of the complexes, by a combination of surface plasmon resonance, isothermal calorimetry and analytical ultracentrifugation.

P-549

Binding of the new actinocine derivative ActII to DNA in the presense of competing ligands

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Actinomycin D is a DNA-binding antitumour antibiotic, but its use suffers from its limited solubility and from causing many side effects. Some actinomycin D derivatives, i.e. new actinocine derivative actii are more soluble than actinomycin D, forms several modes of binding on double-helical DNA but still exhibit anti-cancer activity.
The aim of this study was to investigate the mechanisms of ActII binding to DNA with the help of competing ligands. Competitive binding studies were performed by addition of ethidium bromide (EB), Hoehchst 33258 (HT) and 6-azacytidine (6-AZC), to DNA-bound ActII. A spectrophotometric analysis of competitive binding in isosbestic points of competing ligands was carried out. An analysis of the binding data indicates that ActII and EB, ActII and HT, ActII and 6-AZC compete for the binding sites. Binding parameters of ActIi in presence of competing ligands were also obtained. 6-AZC forms one type of complex with DNA.
Our methodic of analysis of competitive binding in isosbestic points of ligands can be used to obtain binding parameters of different ligands interacting with nucleic acids when they absorb in the same wavelength region. This method can be used also to investigate a complexation in any three – component systems even in the case when one of the competitors has a weak affinity to DNA or does not absorb neither in VIS, nor in UV region.

P-550

In silico approaches to study the associative properties of the small Heat Shock Proteins (sHSPs)

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sHSPs form a superfamily of molecular chaperones with a considerable structural and functional variety. They are able to bind unfolding proteins, but the mechanism of the chaperone function is poorly understood. At the structural level, sHSPs are characterized by assemblies of variable size whilst the monomeric unit is quite conserved within these proteins. Only two structures of this family were solved by crystallography : at a dimer level, the interaction involves a large and flexible loop but interestingly the physico-chemical properties at the interface are different (polar/hydrophobic). These aspects are particularly challenging for protein-protein prediction methods.

We test a protocol to examine various members of the family and the dynamic properties of their subunit (flexibility/specificity). It consists in (1) building a 3D model for the monomer using homology modelling (2) exploring its conformational space by molecular dynamics (3) predicting interaction between subunits by docking approach. Even if the true orientation is missed, preliminary results provide interesting clues about the loop conformation influence in detecting the interface. This study should improve a protocol for docking, which predicts the formation of a complex from homologous proteins and their structures.

P-551

Biophysical characterization of the full-length tumor suppressor protein p14ARF

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INK4a/ARF encodes the potent tumor suppressor protein, ARF, and is the second most frequently inactivated gene in human cancers. ARF inhibits cellular proliferation through protein-protein interactions in partially defined p53-dependent and p53-independent signaling pathways. Although numerous ARF-associated proteins have been identified, few have clearly defined roles in ARF signaling. The most prominent regulators of p53-dependent ARF signaling are Hdm2 and NPM, whose competitive association with ARF either triggers or inhibits p53 activation, respectively. Much less is understood about the p53-independent pathway, although a potential key regulator is the newly discovered protein Abp1, whose protein levels are stabilized upon association with ARF. To date, there is no molecular-based paradigm for ARF's association with its binding partners. The only structural studies of ARF have been limited to interactions between small ARF and Hdm2 peptides, which formed insoluble amyloid-like fibrils upon binding. Such complexes correlate with protein dysfunction; therefore, we hypothesize structural studies of full-length ARF and its partners will provide more biologically relevant insight into the molecular basis of ARF's interactions. We have successfully purified full-length ARF and are using standard biophysical techniques including fluorescence and CD spectroscopy to investigate the structural basis for interactions between ARF and representative binding proteins (Hdm2, NPM, and Abp1).

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– Functional Complexes –

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Crystal structure of lipoate-protein ligase A from Escherichia coli

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Lipoic acid is a cofactor of acyltransferase subunit (E2) of the α-ketoacid dehydrogenase complexes and of H-protein of the glycine cleavage system. It attaches to a specific lysine residue on the proteins via an amide linkage. Lipoate-protein ligase A (LplA) catalyzes the attachment reaction in which a lipoyl-AMP intermediate is formed from lipoic acid and ATP in the initial activation reaction. The lipoyl moiety of the intermediate is then transferred to apopropteins in the second transfer reaction yielding lipoylated protein and AMP. We have determined the x-ray crystal structures of *E. coli* LplA alone and in a complex with lipoic acid at 2.4- and 2.9-Å resolution, respectively. The structure of LplA consists of a large N-terminal domain and a small C-terminal domain. The structure identifies the substrate binding pocket at the interface between the two domains. Lipoic acid is bound in a hydrophobic cavity in the N-terminal domain. No large conformational change is observed in the main chain structure upon the binding of lipoic acid. LplA shows a structural similarity to the catalytic domain of biotin holoenzyme synthetase/bio repressor from *E. coli* whose reaction mechanism is remarkably similar to that of LplA, suggesting that evolutionarily they are closely related.

P-553

Adsorption of amelogenin nanospheres onto charged surfaces towards understanding their auto-assembly

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Self-assembly of amelogenin (rM179) into nanospheres has been postulated to be a key factor in controlling structural organization of the enamel extracellular matrix, which provides the scaffolding for the oriented growth of enamel apatite crystals. Self-assembly was investigated, when nanospheres were adsorbed onto the charged surfaces of auto-assembled polyelectrolyte films (PF). Streaming potential measurements were employed in order to determine the surface charges of nanospheres: they were slightly negatively charged. The buildup of PF's and nanospheres adsorption were monitored in-situ by Optical Waveguide Lightmode Spectroscopy. A monolayer of amelogenin nanospheres was irreversibly adsorbed on the top of a positively charged PF. The temperature dependant experiments showed modifications in the thickness of nanosphere layers confirming the earlier observations made for the nanospheres formation in solution. The local interactions imposed by the adsorption process do not disturb the self-assembling of amelogenin molecules. Multi-assemblies of nanospheres were obtained when mediated by poly-l-lysine and N-Acetyl-D-Glucosamine, known to specifically bind to the three tyrosil motif in amelogenins.

P-554

Mutagenesis analyses reveal distinct roles of TolQ residues for colicin import and ion conductivity

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The Tol-Pal proteins are well conserved in Gram-negative bacteria. This cell envelope system is formed by five proteins located either in the inner membrane (TolA, TolQ, and TolR), in the periplasm (TolB), and anchored in the outer membrane (Pal). Any defect in the Tol-Pal proteins of *Escherichia coli* results in the loss of outer membrane integrity giving cells hypersensitive to drugs and detergents, releasing periplasmic compounds and forming outer membrane vesicles. The Tol-Pal system which transduces the energy provided by the proton motive force of the inner membrane though the periplasm, mediates the interaction between TolA and Pal. Our previous works show that TolQ and TolR transmembrane segments possess topological and sequence homologies with the flagellar motor proteins, MotA and MotB and have suggested that the TolAQR proteins form a molecular motor. In this study, we analyzed the effects of point mutations located in conserved residues of the last two transmembrane segments of TolQ. We demonstrated the importance of glutamate and threonine in channel function as well small lateral chain residues, belonging to the GXXXG motifs, for protein stability or interaction between TolA and TolQ. These point mutations were found to discriminate for the first time residues involved in colicin import or outer membrane stability mechanisms.

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Fluorescence microscopy study of SMAD protein interaction

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Bone Morphogenetic Protein (BMP) signaling occurs via binding of the cell surface receptors consisting of two types of receptor chains BRI and BRII. BMP binding initiates a downstream signal of Smad proteins which regulate the process of cell differentiation and carcinogenesis and mediates the signal into the nucleus. In the resting state Smad proteins are found in both the nucleus and cytosol. After BMP2/4 addition Smad1 becomes phosphorylated, dissociates from the receptor I, binds to Smad4, forming a heterocomplex, and then translocates into the nucleus where the complex associates with other transcription factors and regulates expression of ligand-responsive genes. By fluorescence microscopy techniques, we follow the nuclear translocation of the Smad in cells and report a novel observation of the Smad1/Smad4 complex formation and translocation by FRET imaging. The FRET measured between Smad as fusion proteins with YFP and CFP shows an increasing FRET ratio in cytoplasm immediately after and in the nucleus at longer times after addition of ligand BMP. Translocation into the nucleus Smad proteins after ligand-addition was observed by Laser Scanning Confocal Microscopy and by fluorescence recovery after photobleaching (FRAP) and induced rapid nuclear accumulation of Smad proteins after addition of leptomycin B, an inhibitor of nuclear export, proving constant nucleocytoplasmic shuttling of Smad proteins.

Posters

– Functional Complexes –

P-556

Protein flexibility and entropy on the edge of binding

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Protein recognition puts systems of many thousand interacting atoms on the edge between two conformational states – free and bound. Structure fluctuations may have a profound impact on this process. Yet, flexibility remains until today the weak point in our understanding of protein-protein binding.
We selected a set of 17 protein complexes for which the three-dimensional structures of both free components and the complex were available and performed molecular dynamics simulations in explicit water for each of these 51 systems. Most uncomplexed binding sites proved more flexible than the remaining surface and lost conformational freedom upon complex formation. However, contrary to common expectation, binding did usually not restrict the overall motion of proteins. We calculated the change in conformational entropy from longer simulations on 7 complexes (21 systems) using a new method based on quasiharmonic analysis. Two small complexes and an antibody-antigen system exhibited a significant loss (up to 157$\pm$41 cal/(mol K)) whereas three larger complexes showed increased (by up to 43$\pm$23 cal/(mol K)) or unchanged conformational entropy.
Our results blend in with a unified model of flexible protein recognition [Grünberg et al. (2004) Structure 12, 2125-36] that reconciles the ideas of induced fit and conformer selection. Structure dynamics should influence both the speed of recognition and the stability of protein-protein complexes.

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Promotion of cTAR DNA homodimers by the nucleocapsid protein

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The life cycle of the human immunodeficiency virus (HIV-1) consists of sequential events which are regulated by viral enzymes as well as by cell and viral proteins like e.g. the viral nucleic acid chaperone protein NCp7. The reverse transcription of the single stranded genomic RNA into a linear, double stranded DNA involves two viral proteins: reverse transcriptase and the NCp7 protein. At the beginning, the newly made minus-strand strong stop (ss-cDNA) is transferred to the 3' end of the genomic RNA. This hybridization reaction takes place between regions containing stem-loop structures, so called transactivation response elements (TAR). NCp7 mediates destabilization of both TAR structures in order to chaperone their hybridization. Herein, we have evidenced by two photons FCS that NCp7 promotes formation of homodimers with several cTAR mutants. The NCp7-promoted dimerization of rhodamine-labelled cTAR derivatives has been assessed by the decrease by a factor of two the number of fluorescent species in the presence of protein. These dimers may correspond to kissing complexes or extended duplexes. In addition, homodimers formation was found to be related to the stabilities of the oligonucleotides in their monomeric states. The latter are thought to be of importance in HIV-1 recombination.

P-558

Structural basis of cellulosome efficiency explored by small angle X-ray scattering

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Cellulose, the main structural component of plant cell walls, is the most abundant carbohydrate polymer in nature. To break down plant cell walls, anaerobic microorganisms have evolved a large extracellular enzyme complex termed cellulosome. This megadalton catalytic machinery organizes an enzymatic assembly, tenaciously bound to a scaffolding protein via «cohesin-dockerin» interactions, that enhances synergistic activity among the different catalytic subunits. We have analysed by small angle X-ray scattering and molecular dynamics the solution structure properties of cellulosome-like assemblies. The atomic models, generated by our strategy for the free scaffoldin, and for binary and ternary complexes, revealed the existence of various conformations due to an intrinsic structural flexibility with no or only coincidental inter-cohesin interactions. These results provided primary evidence concerning the mechanisms by which these protein assemblies attain their remarkable synergy. The data suggested that the motional freedom of the scaffoldin allows the precise positioning of the complexed enzymes according to the topography of the substrate, whereas short-scale motions permitted by residual flexibility of the enzyme linkers allow «fine-tuning» of each catalytic domain.

P-559

The Hofmeister effect of anions on the insertion of the antioxidant quercetin in lipid bilayers

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In the last years the interest for Hofmeister effects at the level of cell membranes increased steadily. The salts belonging to the Hofmeister series can have stabilizing or destabilizing effects on cell membrane structures. The Hofmeister anions are frequently encountered in various food products and drugs. For this reason, the study of their effects in membrane structures could contribute not only to elucidating of the mechanisms underlying the function of these structures but also to the development of some applicative domains such as food industry and pharmacology. Our study concerns the effects of some of the anions of the Hofmeister series, especially the nitrite and nitrate ions, on the electrical characteristics of lipid bilayers and of lipid bilayers which contain such flavonoids as the antioxydant quercetin. In order to estimate the electrical parameters of the bilayer, we have used the BLM (black lipid membranes) technique. It has been previously shown that quercetin can insert itself in the artificial lipid bilayers, influencing their electric profile and the insertion depends on the concentration, but also on the composition of the lipid bilayer. It was suggested that the efficacity of quercetin as an antioxydant is correlated to its capacity to incorporate and orientate itself in the lipid plasma membrane. Our aim is to take a step further in this reasearch by studying quercetin insertion in planar lipid bilayers in the presence of several anions of the Hofmeister series.

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– Functional Complexes –

P-560

NMR structural dissection of the human TFIIH transcription factor

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Transcription initiation and nucleotide excision repair (NER) of damaged DNA are connected through the dual action of TFIIH, a multiprotein complex (460 kDa) composed of ten subunits. The combined use of proteolysis experiments and bioinformatics allowed the identification of several structural domains in TFIIH subunits that could be studied in solution using NMR. Among the results of this study, the presence of a new type of C4C4 RING domain within the core subunit p44 is of particular interest[1]. The sequential organisation of β-strands in this RING is related to canonical RING domains by a circular permutation of the β-sheet elements. Another insight into the TFIIH function revealed by this study arose from the identification of a PH/PTB domain within the p62 subunit[2]. We showed that the p62 PH/PTB-like domain is dispensable for the assembly of the TFIIH complex and basal transcription. Instead dual incision experiments revealed that the p62 PH/PTB-like domain is required for nucleotide excision repair and physically interacts with the 3' endonuclease XPG. Thus, our study provides an example of a PH/PTB-like domain potentially involved in the shuttling of TFIIH between NER and transcription.

[1] Kellenberger E. et al. (2005) *J. Biol. Chem.* in press

[2] Gervais V. et al. (2004) *Nat Struct Mol Biol.* **11**(7) 616-622.

P-561

Fluorescence stopped-flow analysis of ClpAPA assembly

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ClpAP is a chaperone-protease complex. It consists of a proteolytic core cylinder (ClpP), which contains several protease active sites and a ring-shaped chaperone complex (ClpA) binding to either end of the core cylinder. ClpA carries ATPase activity and is responsible for recognition and unfolding of protein substrates that then are translocated into ClpP to be degraded. ClpA is a member of the AAA^+ protein family and contains two AAA-cassettes. To perform its biological function ClpA has to form a ring-shaped hexamer. This process is dependent on the binding of ATP. We investigated ClpA hexamer formation, complexation with ClpP and substrate binding using fluorescence methods and rapid kinetic analysis. The time courses were interpreted using numerical fitting procedures.

P-562

DNA- liposome complexes and nuclear pores assembly

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The formation of triple complexes of DNA-liposome-multivalent cations is accompanied by the aggregation and partial fusion of liposomes as we showed by freeze-etching technique. DNA acts as a fusogen in this process, and it unwinds in the region of liposomes fusion. The addition of nuclease S1 to the triple complexes caused a complete fusion of liposomes and the formation of giant liposomes (1-10 mkm in diameter). The addition of DNA, phosphatidylcholine liposomes, and Mg^{++} ions to an egg extract of *Xenopus laevis* lead to the formation in liposomes of structures resembling nuclear pores but of a smaller diameter. Similar structures was shown in liposomes after staining of triple complexes with terbium or lanthanum. By electron microscopy (freeze-etching technique and by Tb^{3+} and La^{3+} staining of triple complexes) we have showed partial fusion of liposomes area, associate with untwisting DNA regions.

DNA –membrane complexes (DMC) in our opinion are the basis of such cellular structures as Bajers junction and mesosomes of bacteria, the nuclear pores and annulate lamellae of eukaryotes, procaryotic and eucaryotic nucleoids. DMC also play certain role in the structure of eucaryotic chromosomes formation and in genome expression.

P-563

Crystallization and structure of a urokinase receptor targeted antibody

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Intervention on urokinase (uPA) proteolytic activity and/or its binding to its receptor, uPAR, has been recognized as a potential mean for anti-tumor metastasis therapy. ATN-615 is a monoclonal antibody that recognizes uPAR at high affinity, and at the same time it does not interfere with uPA binding onto uPAR. The initial crystals of Fab fragment of ATN-615 antibody obtained through vapour diffusion methods diffracted poorly (∼8 Å) with an in-house X-ray source. Treatment of the protein with hydrogen peroxide and reduction of the protein concentration were the key factors in obtaining diffracting crystals. Final crystals diffracted to 1.75 Å, and belong to orthorhombic $P2_12_12_1$ space group with unit cell parameters a=37.2 Å, b=84.5 Å, c=134.0 Å, and contain one molecule of Fab fragment in the asymmetric unit. The antigen binding area (CDR loops) of ATN-165 forms a relative flat and undulating surface, characteristic of anti-protein antibody. It is interesting to note the antigen binding surface is predominately consisted of aromatic residues including tryptophan and tyrosine.

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– Functional Complexes –

P-564

Quantitation of the Ca-dependent interaction of calmodulin with CaM Kinase I and its target sequence

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Calmodulin acts as activator of numerous eukaryotic enzymes, implying close coupling of the binding of Ca and the target protein. We present the quantitation of the affinity of Ca_4.CaM for the CaM-kinase-I protein and its 25-residue target peptide that is fully consistent with the observed enhancement of the apparent Ca-affinity of CaM on complex formation with either ligand.
1) A series of fluorescence based competition titrations establishes the extremely high affinity of the target peptide ($K_d \sim$ pM) in forming the complex (1MXE.pdb : Clapperton et al. (2002) : Biochemistry 41:14669).
2) This peptide affinity is ∼50,000 fold greater than the affinity of the intact kinase for Ca_4.CaM as determined by direct Trp titration; this is rationalised as being largely due to the free energy cost of making available the target sequence in the inhibited intact enzyme.
3) The apparent affinity for Ca binding to CaM is enhanced from pCa 5.3 (CaM) to 6.5 (+ kinase) and 7.4 (+ peptide) [pCa = -$\log_{10}$.Kd(average)].
4) These results, together with the determination of the affinity of either ligand for the Ca-free(apo)CaM, establish the low calcium concentration range over which control of the enzyme activation is exercised in this biological system.
5) Given the compact crystal structure of the inactive enzyme (1A06.pdb), the kinetic mechanism of formation of the Ca_4.CaM.CaM-kinase-I complex appears to be unexpectedly efficient.

P-565

Biophysical study of the interaction between the C-terminal domain of human centrine 2 and Sfi1 repeats

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Centrins, proteins from the EF-hand superfamily, have two main domains, each containing two EF-hand motifs. There are three isoforms in humans, at present under active structural and physicochemical investigation in our laboratory. Some of the calcium binding sites of the centrins have a very low affinity and should remain unloaded in physiological conditions. In human centrin 2 (HsCen2) the active metal binding sites are located in the C-terminal half (C-HsCen2), that undergoes a conformational opening when bound to calcium ions. Several biological functions of centrins are actually known, but incompletely explained. In the microtubule organization centers centrins are critical for the control of normal cell mitosis. This role should involve molecular interactions with other proteins, one of them being the Sfi1. Here we report recent data of our project dedicated to the molecular and structural characterization of the interaction between HsCen2 and human Sfi1 (hSfi1) that has 23 repeats of 23 residues each. We synthesized two different repeats (P1-hSfi1: 611-630, P2-hSfi2: 445-464) and studied their binding capacity to HsCen2, using the ITC technique. While P2-hSfi1 shows no significant binding, probably due to the presence of a Pro in the sequence, P1-hSfi1 interacts with a high binding constant (on the order of 10^7 M^{-1}) and a 1:1 stoichiometry, with the C-terminal half of the protein. We will present the structural and physicochemical properties of the complex C-HsCen2/P1-hSfi1, explored by CD, fluorescence and NMR.

P-566

Evidence for glycolytic enzyme complexes in rabbit myoplasm based on stoichiometry and diffusivity

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Glycolysis is important in fast twitch muscles, where ATP, produced at intermediate steps of the glycolytic pathway, fuels contraction and a variety of other ATP-dependent cellular processes. It has been postulated that glycolytic enzymes form complexes *in vivo* that facilitate metabolic channeling of intermediates, but direct evidence remains elusive. To probe the properties of the putative *in vivo* complex, we measured the concentrations and efflux of glycolytic enzymes from single demembranated muscle fibers transferred from oil through several solution drops in which diffusible protein content was measured. Molar concentrations for enzymes catalyzing the hexose and triose sugar reactions grouped around 40 μM and 80 μM, respectively, consistent with a stoichiometry of ∼1:2 that suggests a basis for metabolic channeling. Efflux rates increased significantly with protein depletion, with final values for the diffusion coefficients inversely proportional to hydrodynamic radii and roughly a tenth that in bulk water. We speculate putative complexes break apart as the pool of monomers becomes depleted. As individual enzymes diffuse radially outward they pass through a molecular sieve of effective mesh size ∼15 nm, i.e., comparable to the interfilament spacing of the I-band lattice.

P-567

sHSP properties of alpha-crystallins investigated with Quasi Elastic Light Scattering

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Small heat shock proteins (sHSP) form a distinct family of molecular chaperones with a highly conserved C-ter domain, around 90 aa, "the α-crystallin domain", and a variable N-ter. They form large oligomeric complexes, 12 - 40 subunits of 12 - 43kDa and rapidly exchange subunits. In eye lenses, αA and αB crystallins are combined to form high molecular weight polydisperse oligomers (800kDa) that ensure lens transparency. In the chaperone-like function, subunit exchange allows them to associate β- and γ- crystallins at the onset of their denaturation thus preventing aggregation and cataract. Recently, Fluorescence Resonance Energy Transfert and Small Angle X-ray Scattering were used to study the formation of α-crystallin / substrate complexes as a function of temperature [1]. In the present study, Quasi Elastic Light Scattering was used to compare oligomeric state, subunit exchange and transition temperature of native α-crystallins and of a variety of mutants, including the R120G human α*B*-crystallin, responsible for a myopathy and a cataract [2]. The kinetics of the conformational transitions and of the α-crystallin / substrate complex formation were followed in addition.
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Posters

– Functional Complexes –

P-568

Visualization of single *Escherichia coli* FtsZ filament dynamics with atomic force microscopy

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FtsZ, the prokaryotic homologue of tubulin, is an essential cell division protein. In the cell it localizes at the center forming a ring that constricts during division. *In vitro* it binds and hydrolyzes GTP, and polymerises in a GTP-dependent manner. We have used atomic force microscopy (AFM) to study the structure and dynamics of FtsZ polymer assembly on a mica surface under buffer solution. The polymers were highly dynamic and flexible, and continuously rearranged over the surface. End-to-end joining of filaments and depolymerization from internal zones were observed, suggesting that fragmentation and reannealing may contribute significantly to the dynamics of FtsZ assembly. The shape evolution of the restructured polymers manifested a strong inherent tendency to curve. Polymers formed in the presence of non-hydrolysable nucleotide analogues or in the presence of GDP and AlF_3 were structurally similar but showed a slower dynamic behavior. These results provide experimental evidence supporting the model of single-strand polymerization plus cyclization recently proposed to explain the hydrodynamic behaviour of the polymers in solution.

P-569

An electrical bio-sensing platform using micro-cantilevers

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A microfabricated cantilever with an internal piezoresistive element has been used for *electrical* detection of two types of interactions, DNA-DNA hybridization, and recognition between oestrogen receptor (ligand bound/free) and conformation specific peptides α/βI (recognises oestradiol bound receptor), and α/βII, (recognises ligand free receptor). Generation of a differential surface stress upon target binding allows identification of 12 nt DNA probes with *point mismatch discrimination* (sensitivity 200 nM). An overhang extension, however, enhances sensitivity 10 fold. Multiplexed analysis of a range of target probes using different sensor probes is performed *in situ* in real time. Replacing the capture probe with locked nucleic acid (LNA) results in faster target capture kinetics compared to DNA. Influence of steric and electrostatic factors on signal generation is evident in signal enhancement by the overhang extension and reversal of signal polarity by varying buffer strength, respectively. Ligand induced *protein conformational changes* is detected by discrimination between oestradiol bound and free forms of two proteins, oestrogen receptor ligand binding domain (ER-LBD) and glutathione *S*-transferase bound ER-LBD at a range of protein concentrations (2.5-20 nM) and ligand concentrations (125-1000 nM). A direct detection compared to the optical method this assay offers integrated readout, label-free and non-invasive communication with a range of functional complexes at solid-liquid interfaces.

P-570

Structure of human T-protein of glycine cleavage system: Implications for nonketotic hyperglycinemia

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T-protein, a component of the glycine cleavage system, catalyzes the formation of ammonia and 5,10-CH_2-H_4folate from the aminomethyl moiety of glycine attached to the lipoate cofactor of H-protein. Several mutations in the human T-protein gene cause non-ketotic hyperglycinemia. To gain insights into the catalytic mechanism and the effect of disease-causing mutations at the molecular level, crystal structures of human T-protein in free form and bound to 5-CH_3-H_4folate have been determined at 2.0 Å and 2.7 Å resolutions, respectively. It comprises three domains arranged in a cloverleaf-like structure with the central cavity, where 5-CH_3-H_4folate is bound. Most of the disease-relating residues cluster around the cavity forming hydrogen bond networks to hold not only the folate-binding space but also the positions and the orientations of the second structural elements in the middle segment, which might play a pivotal role in the T-protein catalysis. The catalytic residues directly participate in C_1 unit transfer was verified by the mutation analysis, and the complex model of H- and T-protein provides a reasonable picture of the catalytic process, showing an ingenious mechanism of recruiting the aminomethyl lipoate arm to the reaction site without the release of toxic formaldehyde.

P-571

Calmodulin binding to the MAGUK family of synapse-associated proteins

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Membrane-associated guanylate kinase (MAGUK) are multidomain proteins, encompassing at least one PDZ domain, an SH3 domain, and a guanylate kinase (GK)-like domain. The subfamily comprising the synapse-associated proteins (SAPs) SAP90/PSD-95, SAP97/hDlg, SAP102/NE-Dlg, and PSD-93/Chapsyn-110 contain three amino-terminal PDZ domains; CASK and its homologs have a calmodulin-dependent protein kinase (CaMKII)-like domain at the N-terminus; the zonula occludens proteins ZO-1, ZO-2, and ZO-3 have an extended C-terminal region; p55 and other members of the fourth subfamily consist mainly of the three core domains. Different modes of inter- and intramolecular interactions are proposed to occur between the SH3 and GK domains and the so-called HOOK region located between these two domains. The GK domain is devoid of enzymatic activity; it appears to have evolved as a protein-protein interaction module that associates with a novel class of proteins designated GKAP. Comparison of the 1.3 Å structure of the GK domain of human CASK with the structures of yeast GMP kinase shows important differences in the GMP binding site. By using surface plasmon resonance spectroscopy we characterized the high affinity (K_d of 50 to 200 nM) interaction of calmodulin with various MAGUKs, the HOOK region being of critical importance for complexation. Our findings suggest that calmodulin could act as a trigger molecule to switch MAGUKs from a closed to an open conformation where binding sites are unmasked.

Posters

– Functional Complexes –

P-572

Hair: Molecular rearrangement during growth and development leads to tough resilient fibres

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The axial arrangement of IF molecules in hair has been determined. The key to success was our ability to reconstitute expressed mouse keratin type I and type II IF chains into filamentous structures identical to those observed *in vivo.* DST crosslinks were introduced between axially adjacent lysine residues in different molecules within the filaments. These peptides were sequenced and the interacting lysine pairs determined for intermediate filaments in both an oxidising and a reducing environment. Remarkably, whilst most of the molecular pattern of interactions was identical in these two cases there was one significant difference between them, and this corresponded to an axial shift between molecules. This allowed cysteine residues to come into axial alignment and to form covalent disulphide bonds.

At the base of the hair follicle the intermediate filament proteins are expressed in a reducing environment, and the molecules assemble into hair filaments. Further up the hair follicle the matrix proteins are laid down and these interdigitate between the filaments. Cell death occurs and the environment changes to an oxidising one. The filaments undergo a structural rearrangement (the first ever seen in a fibrous protein) to permit disulphide bonds to be formed within the intermediate filament itself, thus endowing it with exceptional mechanical strength. There are thus two unique structures adopted by hair intermediate filaments, depending on the stage of development.

P-573

Involvement of the projection domain of MAP4 in the regulation of the microtubule dynamics

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Individual microtubules (MTs) undergo transitions between phases of polymerization and depolymerization, so-called MT dynamic instability. The heat-stable MAPs (MT associated proteins) including MAP4, MAP2 or tau are known to regulate the dynamic instability. These MAPs possess a common asymmetric structure consisting of N-terminal projection (PJ) domain and C-terminal MT-binding (MTB) domain. The PJ domain has not been thought to participate in the MT dynamics, because it does not bind MT directly but protrudes from the MT surface. To investigate the role of the PJ domain in MT dynamics, we observed the dynamic instability by dark-field microscopy in the presence of several mutants of MAP4 with different deletions of the PJ domain. Depending on the deleted regions, the dynamic instability was attenuated by decrease in both polymerization and depolymerization distances. Additionally, profiles of the dynamic instability were quite different in the presence of PJ2, a MAP4 mutant lacking the 2/3 of N-terminal part of the PJ domain. Relatively rapid and long depolymerization phases were sometimes observed among quite slow length changes. Fluorescence microscopic observation suggested that the different profiles were due to the inhomogeneous distribution of PJ2 along the MT lattice. Taken together, we conclude that the PJ domain participates in the regulation of the MT dynamic instability.

P-574

Ethanol induced changes in HDL oxidation studied by non-radiative energy transfer fluorescence

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The aim of the study was to follow the influence of alcohol on the structure and function of serum high density lipoproteins, HDL. The effect of different concentrations of ethanol on the HDL oxidation process was monitored by fluorescence spectroscopy of apoA-1 tryptophans and molecular probe 6-propionyl-2-dimethilaminonaphtalen, PRODAN. PRODAN, although predominately lipophilic, participates in environments of different polarity, in HDL monolayer and in water media. The mathematical model for calculating the spectra of PRODAN only in lipid matrices of HDL was developed. The changes provoked at lipid/protein interface, at different stages of particle oxidation, were studied by non-radiative energy transfer, NRET. The concentration of ethanol and the stage of HDL oxidation modulate the process of energy transfer. The increase of ethanol concentration disrupts the communication over the boundary between protein and lipid monolayer as seen by NRET. The process of oxidation is slowed down with the increase of ethanol concentration. In fully oxidative state HDL is more sensitive to the ethanol induced perturbation.

P-575

Mapping of interface residues of Pla protein from Y. pestis with Plasmin(ogen) by MD simulations

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The plasmin(ogen) system (Plg) is an important mechanism for the cell migration through the tissues in the mammalian organisms. Some bacterial agents can activate this system by proteases and lead an uncontrolled degradation of extracellular matrix components, and make an invasive character of these infections. The *Y. pestis* protein Pla is a plasmid coded outer membrane protein, with aspartic-protease activity and is closely related with the proteolytic activation of Plg in the serine-protease form called Plasmin. Exactly how the Pla activate Plg in plasmin remains unclear. For better understand about this event, we performed in this work the predicted interaction between the Plg and Pla protein by docking and evaluate the complex stability by Molecular Dynamics (MD).The predicted complex of Plg-Pla show same interaction site predicted by experimental site direct mutagenesis in others studies. After 8 ns MD (72083 atoms in simulation box), we observed the relax of beta barrel structure of Pla and the progressive approximation between mass center of this molecules, followed by the increase of hydrogen bonds number and total contact area, mainly of hydrophobic residues. The mapping of this interface region after simulations shows the participation of 30 aminoacids in Pla and 30 in Plg. In this study we report the possible aminoacids that can be participant in the active site and the sub sites of interaction. The total understanding of these interactions can be a important tool for drug design against bacterial proteases.

Posters

– Functional Complexes –

P-576

Structure of the R-state of E.coli aspartate transcarbamoylase with one substrate bound

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E. coli aspartate transcarbamoylase (ATCase) catalyzes the first step in pyrimidine biosynthesis, condensation of carbamoyl phosphate (CP) and aspartate (ASP) to form N–carbamoyl-L-aspartate (CA) and inorganic phosphate (P_i). In the wild-type enzyme binding of ASP to the E•CP complex induces domain closure in catalytic chains, which induces dramatic quaternary structural change from inactive T to active R structure. One interaction stabilizing the T state of the enzyme is between Asp236 on the catalytic chain to Lys143 on the regulatory chain.The structure of the D236A mutant was investigated by SAXS and X-ray crystallography with PAM, an analogue of first substrate CP, to understand how this mutation can destabilize one of the allosteric states of ATCase and trap the enzyme in the R quaternary structure with half of the active site unoccupied. SAXS experiments showed the enzyme remains in the R state not returning to the T state until the supply of substrates is exhausted. Therefore, the D236A structure corresponds to the activated state of the enzymatic cycle when CP has bound and the binding site for ASP is formed, but unoccupied. The D236A/PAM complex analysis utilizing *in silico* docking experiments and molecular dynamic simulations reveals that the architecture of the active site cavity of ATCase is altered during the enzymatic catalytic cycle.

P-577

The nDsbD-SS-CcmG complex from E.coli: structural basis for DsbD-dependent cytochrome c maturation

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Escherichia coli DsbD transports two electrons from cytoplasmic thioredoxin to substrate proteins (DsbC, DsbG, and CcmG) in the periplasm. DsbD is composed of a N-terminal periplasmic domain (nDsbD), a C-terminal periplasmic domain and a central transmembrane domain. Each of these domains possesses two cysteines, which are required for the electron transport. Fast (3.9×10^5 $M^{-1}s^{-1}$) and direct disulfide exchange occurs between nDsbD and CcmG, a highly specific disulfide reductase essential for cytochrome *c* maturation. We solved the crystal structure of the disulfide-linked complex between nDsbD and the soluble part of CcmG at 1.94 Å resolution. In contrast to the other two known complexes of nDsbD with target proteins, the N-terminal segment of nDsbD contributes to specific recognition of CcmG. This and other features, such as the possibility of using an additional interaction surface (like in the disulfide-linked complex with DsbC), constitute the structural basis for the adaptability of nDsbD to different protein substrates.

P-578

Fast assignment of NMR backbone resonances for proteins of known 3D structures using RDCs

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Structural genomic projects today yield an increasingly high number of proteins structures, mainly obtained by X-Ray diffraction, whose functions remain to be elucidated. NMR can play here a crucial role, through its ability to quickly identify binding sites of ligands on proteins, by measuring the evolution of chemical shifts on fast and sensitive HSQC type experiments (Chemical Shift Mapping). An important NMR limiting step is the assignment of the HSQC spectra, which remains often fastidious, and big efforts are being made to provide to the NMR community automated assignment methods. To be applicable to high throughput procedures, the methods need to be fast and reasonably priced, and thus to use (i) a minimal amount of preferably only ^{15}N-labeled protein, (ii) fast, highly sensitive, and generally practicable experiments.

For proteins whose 3D structures alone are already known, the comparison of back-calculated with experimental Residual Dipolar Couplings (RDCs) values can be a very efficient way to obtain a straightforward assignment of HSQC spectra. It is however in practice not sufficient, and a number of methods have been proposed that use additional information such as H^N-H^N nOes, chemical shifts, accessibility of amide protons, etc.

Here, we propose a new approach that exploits RDCs to get a fast and automated assignment of interaction sites in proteins of known 3D structures. We also analyze in detail the theoretical and experimental limits of the use of RDCs in this context.

P-579

Isolation, characterisation and crystallisation of a new AAA-protein from archeae

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A new member of the AAA (ATPases Associated with various cellular Activities) protein family existent in certain archaeal species has been analysed in our work.

The genes of Archaeoglobus fulgidus and Methanosarcina acetivorans have been cloned from genomic DNA and were inserted into protein expression vectors. The monomeric weight of the A. fulgidus AAA-protein is 39.9 kDa and the monomeric weight of M. acetivorans AAA-protein is 42.2 kDa. Proteins were purified to homogeneity as verified by SDS-page and sedimentation velocity analysis. The assembly state was shown to be hexameric by sedimentation equilibrium measurements over a wide concentration range. Electron micrographs show homogeneous, ring shaped hexamers with 100-130 Å diameter. As suggested by homology to members of the AAA+ family, this AAA-protein shows ATPase activity.

Posters

– Functional Complexes –

P-580

Formation and function of giant gel network in marine ecosystem

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The mechanism of marine gel formation, its stability and the role of gel state in marine ecosystem is becoming a most challenging exercise in converging disciplines of marine chemistry, microbiology and biophysics.
The enigmatic gel phase appears episodically in the northern Adriatic Sea. The phenomenon manifests itself in rapid production of enormous amounts of gelatinous matter in the water column and on the sea surface. Current views leave no doubt on phytoplankton as a proximal source of polymers constituting the gel network, but the mechanism leading to its rapid formation remains unknown. Our biophysical scenario of gel formation features self-organization of biopolymers into marine vesicles that transform to giant-gel by the first order phase transition.
We introduced electrochemical sensing of marine vesicles and AFM to image supramolecular organization of native gel network. Gel structure exhibits a repeating network of solvent cavities (150-500 nm) between entangled rigid fibrils with 0.6-3 nm in diameter. The giant gels function as efficient bioreactors to eliminate excess of photosynthesized material from the basin and restore normal populations in the microbial community.

P-581

Complex between triple helix of collagen and double helix of DNA in aqueous solution

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We demonstrate in this paper that one of examples of biologically important and molecular self-assembling complex system is a collagen-DNA ordered aggregates spontaneously formed in aqueous solutions. Interaction between collagen and DNA lead to destruction of hydration shell of triple helix and to stabilization of the double helix structure. From the point of view of molecular biology this nano-scale self-assembling superstructure can determine the raising of the stability of DNA against the nucleases during the collagen diseases, growth of collagen fibrils in the presence of DNA and indicates a development of autoimmune reaction. In addition, collagen is one of the most useful carrier materials for new drug and gene delivery system. Usually DNA remains as a giant molecule packed in the cells nucleus and is composed of proteins in the chromatin fibers. However, during some pathological processes, in particular, during collagen diseases, DNA is released from the nucleus and is placed in the matrix between the cells – in the connective tissue. Our molecular model suggests that DNA, containing well arranged phosphate groups, helps the collagen to make fibrils by water molecules. Such extraordinary complex induces distraction hydration shell of collagen triple helix, stabilization hydration shell of ds-DNA, stability of DNA against the nucleases and this complex between collagen and DNA determines unusual properties of collagen fibers.

P-582

Interaction of bovine seminal plasma protein PDC-109 with phospholipids and soluble ligands. A spectroscopic study

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The major protein of bovine seminal plasma, PDC-109, selectively interacts with choline phospholipids on sperm plasma membrane and stimulates *cholesterol efflux*, a critical step in sperm capacitation. Previous ESR and surface plasmon resonance studies indicated that PDC-109 penetrates into the membrane interior upon binding, and that its interaction with phosphatidylcholine is characterized by faster association and slower dissociation rate constants as compared to other phospholipids. Here the interaction of PDC-109 with phosphorylcholine (PrC) and choline phospholipids is investigated by absorption, ^{31}P-NMR and fluorescence spectroscopy. Binding of PDC-109 to DMPC induced the formation of an isotropic signal in ^{31}P-NMR, which increased with increasing protein/lipid ratio and with increase in temperature. Addition of cholesterol reduced the isotropic signal, indicating the stabilization of the lamellar phase. Quenching of the protein fluorescence by acrylamide and iodide was considerably reduced when PDC-109 was bound to DMPC vesicles, indicating a partial penetration of the protein into the membrane interior, resulting in a shielding of tryptophan residues. Absorption titrations indicate that lyso-PC ($K_a = 2 \times 10^4$ M^{-1}) binds to the protein with a 250-fold higher affinity over PrC ($K_a = 81$ M^{-1}). Thermodynamic parameters, ΔH^o and ΔS^o, indicate that a smaller negative entropy of binding for Lyso-PC results in its significantly stronger binding.

P-583

Energetics of GrpE·DnaK binding. Role of ATPase and substarte binding domains

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Molecular chaperones mediate essential intracellular processes (folding of nascent polypeptides, assembly of proteins and dissasembly of protein aggregates) under physiological and stress conditions. The *E. coli* Hsp70 chaperone, DnaK, acts as a nucleotide-activated molecular switch controlled by the action of the cochaperones DnaJ and GrpE. GrpE regulates nucleotide (ADP/ATP) exchange and unlocks the lid subdomain, favoring substrate dissociation.
We have characterized the energetics that govern complex formation between GrpE and DnaK and the contribution of specific DnaK domains to the binding reaction using different mutants: the deletion mutants DnaK(1-385) and DnaK(1-507), the single point mutant DnaK D526A and the double point mutant DnaK D540A K548A. Comparing the results obtained by high-sensitivity titration microcalorimetry, we can disect the contribution of each DnaK domain to the stability of its complex with the nucleotide exchange factor GrpE.

Posters

– Functional Complexes –

P-584

Crystal structure of inhibitor-bound mouse cytidine deaminase

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Cytidine deaminase (CDA) catalyses the deamination of (deoxy)cytidine to (deoxy)uridine. Two types of CDA, dimeric and tetrameric CDAs, have been classified [1,2]. The dimeric CDA has two cysteine and one histidine residues liganding a zinc ion at the active site, whereas the three residues are all cysteine in the tetrameric CDA. Arg56 of the tetrameric CDA from *Bacillus subtilis* partly neutralises the negative charge of the cysteine [3].
The inhibitor-bound structure of the tetrameric mouse CDA has, surprisingly, revealed the corresponding residue, Arg68, in two alternate conformations. While in the first conformation Arg68 forms hydrogen bonds with two of the zinc-binding cysteine residues, in the second conformation these hydrogen bonds are abolished. Although hydrogen bonds are important for maintaining zinc reactivity [3], the absence of it in the second conformation can conversely facilitate product dissociation by increasing negative charge donation from cysteine to the zinc ion, hence weakening the zinc–product interaction. Furthermore, the nearby Gln72 dyad, formed by Gln72 from two adjacent subunits, interacts with Arg68 in the second conformation, suggesting an allosteric cooperativity between the two subunits.
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P-585

Catalytic mechanism of bisphosphoglycerate mutase revealed by substrates binding mode

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Bisphosphoglycerate mutase (BPGM EC 5.4.2.4) can catalyze three reactions: synthase, mutase and phosphatase, with the main function is to synthesize 2,3-diphosphoglycerate (2,3-BPG), the allosteric effector of hemoglobin. The regulation of 2,3-BPG metabolism is of particular interest because this compound profoundly influences the affinity of hemoglobin for oxygen in vertebrates. The issue of the substrates binding pathway and mechanism remains a subject of intense debate. 2,3-BPG and 3-PGA binding modes were obtained by cocrystallization BPGM and these two substrates. Reaction proceeds during the crystal growth contributing to different ligands binding at the active site with significant conformational change, giving hints to molecule mechanism of enzyme. The mechanism is based on the phosphorylated catalytic residue His11 and several other key residues in the active site. Phosphorus intermediate was formed by associative pathway.
Keywords: bisphosphoglycerate mutase, complex structure, mechanism

P-586

Interaction analysis of MARCKS PSD domain with its targets by ESI-MS

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Myristoylated alanine-rich C kinase substrate (MARCKS) interacts with Ca^{2+}-calmodulin, actin, and acidic phospholipids through phosphorylation site domain (PSD), and these interactions are regulated by each other and by N-terminal myristoylation and phosphorylation. We have revealed that the PSD domain interacts with calmodulin in unique elongated conformation by X-ray crystallography (Yamauchi E *et al*. Nat Struct Biol., 10(3), 226(2003)).
In this study, we have synthesized several peptides derived from MARCKS PSD domain, and examined the region of MARCKS involved in actin, phospholipids, and calmodulin binding by electrospray mass spectrometry using hydrogen/deuterium (H/D) exchange method. The H/D exchange rates of the PSD peptide bound to its targets were different from that observed with free peptide, suggesting that the peptide was partially masked from solvent by the interaction with the targets. In addition, the H/D exchange rates were different from each other depending on the bound target. The interacting residues were also studied by MS/MS analysis. The results suggest that the multi-functional PSD domain adopts its various target using different interactions.

P-587

Aspirin binding site in human serum albumin revealed by crystal structure of their complex

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Human serum albumin (HSA) is an abundant transport protein in plasma that can bind a wide variety of endogeneous compounds and drugs in two primary binding sites (I and II) and plays a key role in drug's pharmacokinetics. Aspirin is a widely used drug for nonsteroidal anti-inflammatory and anti-thrombosis application. The structure of the complex between HSA and aspirin has not been described in detail. Here we report the crystal structures at 2.7 Å resolution of HSA-aspirin complex in the presence of myristate. The structure demonstrates that aspirin binds only to drug site I (in subdomain IIA) of HSA but not to the drug site II (in submain IIIA) in the presence of fatty acids. The carboxyl group of aspirin makes polar contacts with Arg-222, his-242, Lys-199 of HSA. The phenyl ring of aspirin occupies but does not fill up a hydrophobic pocket in HSA consisting of Phe-223, Trp-214, Arg-218, Leu-238, Ile-264, Ile-290, Ala-291.

Posters

– Functional Complexes –

P-588

Functional selectivity of Src Homology 3 domains by tertiary interactions

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Eukaryotic signalling events and cytoskeletal arrangements involve the recognition between protein-protein interaction modules and their cognate binding motifs. Revealing the selection mechanisms that allow unequivocal ligand recognition is central to both understanding cellular function and conceptualising therapeutic intervention. Src Homology (SH) 3 domains are ubiquitous signalling domains. However, from their structure and affinity towards their cellular ligands (proline-rich motifs), they do not seem to offer enough selectivity to guarantee non-erroneous signalling. Combining crystallographic analysis, Small Angle X-ray Scattering, Isothermal Titration Calorimetry and cellular biology, we reveal the occurrence of supplementary (so-called tertiary) interactions in cellular signalling, and illustrate, based on the example of the PI3 kinase, how these tertiary interactions can introduce a level of 'functional' selectivity.

P-589

Binding of cationic porphyrin to isolated double-stranded DNA and nucleoprotein complex

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The complexation of tetrakis(4-N-methylpyridyl)porphyrin (TMPyP) with free and encapsidated DNA of T7 bacteriophage was investigated. To identify binding modes and relative concentrations of bound TMPyP forms, the porphyrin absorption spectra at various base pair/porphyrin ratios were analyzed. Spectral decomposition, fluorescent lifetime, and circular dichroism measurements proved the presence of two main binding types of TMPyP, e. g., external binding and intercalation both in free and in encapsidated DNA. TMPyP binding does not influence the protein structure and/or the protein – DNA interaction. Concentrations of TMPyP species were determined by comprehensive spectroscopic methods. Our results facilitate a qualitative analysis of TMPyP binding process at various experimental conditions. We analyzed the effect of base pair composition of DNA, the presence of protein capsid and the composition of buffer solution on the binding process.

Posters
– Modelling Molecules –

P-590

Molecular simulations study of the C12E4 reverse micelles changes with the temperature and pressure

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Molecular Simulations (MD) in NPT ensemble at T=8 and 25°C, P=0.1 and 200 MPa have been performed to investigate the structural changes of small size nonionic ($C_{12}E_4$) reverse micelles (RM) in decane. Experiments suggest that the Ethylene-Oxide polar heads (Eph) of the $C_{12}E_4$ adopt a helical conformation in water.We have thus built 2 types of RM:all-trans and helix conformers for the Eph. For each T and P,we examined the RM structure variation with time: the water core and of RM sizes, the radial profiles, the Eph hydration, the $C_{12}E_4$ conformation etc.Our MD shows that RM spherical-like shapes and sizes do not significantly depend on T. For the RM with the Eph in gauche conformation, we observe a significant dehydration of the Eph when T increases,in contrast to the all-trans conformation.For all simulated RM, a small number of transitions of the Eph dihedral angles have been observed. When P increases, we observe a small reduction of the RM and water core radii; whereas the Eph hydration increases.The rigidity of the Eph is found close that of the micellar water. This value is larger than the rigidity of the C_{12} aliphatic chain,close to the decane rigidity.Our MD indicates that low water content small RM decreases significantly the response of aggregates and $C_{12}E_4$ to P and T;in contrast to larger micelles.Alternative MD methods (for ex. in implicit solvent) are needed to gain more information about this ternary system.

P-591

Exotic solitons in a discrete model for DNA denaturation

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We analyse the appearance of some exotic soliton like solutions named as compactons, cuspons, etc. for the precursors of the denaturation in a certain generalization of the model proposed by M. Peyard and A.R. Bishop[1]. This discrete nonlinear evolution equation takes into account inharmonic interparticle interactions between sites. In our model we considered two degrees of freedom for a DNA chain. The Morse potential is taken as the average potential representing the two or three bonds which connect the two basis in a pair. The new obtained solutions are results of crucial restrictions concerning the main model parameters, the boundary conditions and the application of the method of effective potentials. Their main dynamic characteristics are calculated and the influence of these structures in the appearance of the denaturation of the model of double fiber as a result of the power concentration is studied.
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P-592

Self-assembly of collagen: A model for inhomogenously charged biomolecular aggregation

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A standing question in biological systems is how biomolecules with highly inhomogenous charge distributions aggregate to form superstructures such as amyloids, protein complexes, or structural assemblies in living systems. A perfect test case for the understanding of these sorts of processes is that of collagen self-assembly. Collagen, an inhomogenously charged rodlike monomer, can aggregate *in vitro* into a variety of supermolecular structures that have characteristic periodic features. This assembly process appears to occur as a function of pH, temperature, counterion strength and identity, but importantly does not require the presence of co-factors or other complicated biomolecules. Due to the relative simplicity of the experimental parameter space and the periodic topology of the aggregates, the assembly process can be modeled using a variety of theoretical tools. We present first a full three-dimensional all-atom model for the collagen monomeric protein that has been refined using molecular mechanics and dynamics simulations using the CHARMM forcefield. We further present applications of this model that address important experimental observations related to the formation of the most common collagen structures found *in vivo* and *in vitro*. In the context of these observations we suggest some general approaches to understanding the self-assembly of highly charged biomolecules.

P-593

Chromatin flexibility simulated by a Monte Carlo model

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For a zig-zag type model of the 30 nm chromatin fiber, we estimate basic structural and physical properties such as the persistence length and stretching elasticity. The data was obtained from Monte-Carlo (MC) simulations of the stretching of a single chromatin fiber on a computer. The model approximates the DNA by a flexible polymer chain with Debye-Hückel electrostatics and uses a two-angle zig-zag model for the geometry of the linker DNA connecting the nucleosomes. The latter are represented by flat disks interacting via an attractive Gay-Berne potential. Our results show that the stiffness of the chromatin fiber strongly depends on the linker DNA length. Furthermore, changing the twisting angle between nucleosomes form 90° to 130° increases the stiffness significantly. The simulated persistence lengths and elastic moduli agree with experimental data. Most importantly, we show that the chromatin fiber does not behave as an isotropic elastic rod, but its rigidity depends on the direction of deformation: chromatin is much more resistant to stretching than to bending.

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– Modelling Molecules –

P-594

Theoretical description of the hydration shell of proteins

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The protein structure is strongly determined by the interaction with the solvent. A change in the physical parameters of the surrounding water can induce changes in the shape of its molecule or/and biological activity. It is expected that some interesting information on the character of water-biomolecule interactions can be extracted from a study of the effect of high pressure. The density of water in the hydration layer is much greater than in the bulk, moreover, the water molecules are oriented and crowded on the surface of the protein molecule in the vicinity of charged groups. The density of hydration water is proportional to the electric field generated by atomic partial charges. The compression is due to the pull of the dipoles of H_2O molecules, necessary to achieve the thermodynamic equilibrium, from bulk water into the high field region at the surface of the protein molecule. As follows from our estimations, the electric field intensity on the lysozyme surface reaches a value sufficient to perform work of electrostriction. The properties of water in such strongly compressed hydration shell are much different from those in the bulk. The anticipated changes in the density and permittivity of the hydration layer under the effect of hydrostatic pressure or temperature are correlated with phase transitions of the proteins.

P-595

NMR of symmetric dimers and NOE assignment

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The interactions between biomolecules are playing a major role into the organization of biological systems. The understanding of these interactions at the atomic level is thus mandatory, and is requiring the determination of the complexe 3D structures or of the interaction surfaces.
The Nuclear Magnetic Resonance (NMR) structure determination of homo-dimers is facing to the assignment of the inter- and intramolecular NOEs. The method ARIA (Habeck et al., 2004) for the automatic assignment NOEs is being modified to be used in the case of homo-dimers. Examples will show that homodimeric structures can be obtained and corresponding NOEs automatically assigned, while keeping the same level of structure quality than that observed for monomeric structures.
M Habeck , W Rieping, JP Linge and M Nilges. NOE assignment with ARIA 2.0: the nuts and bolts. (2004) *Methods Mol Biol.* **278**, 379-402.
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P-596

GDNF receptors: Veterans vs Novices

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The GDNF (Glial cell line-Derived Neurotrophic Factor) family of growth factors presently consists of four proteins: GDNF, neurturin (NRTN), persephin (PSPN) and artemin (PSPN). They have all been shown to act as potent neuronal survival factors, but at least GDNF has several functions outside the nervous system.
The receptor complex consists of the signalling module, a tyrosine kinase termed Ret, and a cell surface bound co-receptor, the GDNF family receptor alpha (GFRa). Upon activation, this complex promotes cell survival, neurite outgrowth, cell differentiation and other processes.
Recently, we solved the structure of GFRa1 (the GDNF co-receptor) domain 3 at 1.8 Å resolution showing a new protein fold. It is an all-alpha five-helix bundle stabilized by five disulfide bridges. The structure was used to model the homologous domain 2, the other half of the GDNF-binding fragment, and to construct the first structural model of the GDNF-GFRa1 interaction. The model has been confirmed by site-directed mutagenesis.
In addition, we found that GDNF, ARTN and NRTN use alternative receptor system based on heparan sulphate proteoglycans termed Syndecans. This receptor system could only be activated when GDNF was presented as matrix protein thus highlighting the functional asymmetry of the cellular response to the growth factor.

P-597

Application of molecular modeling to analysis of protein-ligand interactions

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Molecular modeling approaches such as docking and molecular dynamics (MD) are commonly used to study protein-ligand interactions. These methods have potential applications in studying enzyme substrate specificity and in identifying protein inhibitors, as is done in rational drug design. Presented here are some examples from our lab in which modeling has been applied to study protein structure and function. In studies of β-glucosidases, homology models of all family 1 enzymes in *Arabidopsis thaliana* were generated. Analysis of the binding site and application of molecular docking provided insights into substrate specificities of these enzymes. In addition, molecular docking and MD were applied to studies of inhibition of the human motor protein denoted HsEg5 and other homologues in the BimC subfamily, which are essential for mitosis. Using the crystal structure of human Eg5 as a template, homology modeling was used to generate models of the *Xenopus, Drosophila,* and *Aspergillus* homologues. A series of known inhibitors was docked into each of the homologues, and the differences in binding energies were consistent with reported experimental data. Molecular dynamics revealed significant changes in the structure of the *Aspergillus* homologue that may contribute to its relative insensitivity to inhibitors of human Eg5.

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– Modelling Molecules –

P-598

Hydration of Z-RNA stabilizes the left-handed helix: A computational study

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The Z-RNA structure solved by Popenda, Milecki and Adamiak (Nucleic Acids Res. 2004, 32, 4044) was used as the model for molecular dynamics simulations in aqueous solution and DFT calculations. Their objective was to understand the modes of stabilization of the Z-type left handed duplex in solution. Hydration patterns in both major and minor groove were investigated. The particular hydration network around the cytidine ribose hydroxyls was described and a model was proposed on how water contributes to their interactions probably playing an important role in the overall Z-RNA duplex conformation. This network was further studied by the ONIOM method. Also the potentially important hydration sites in the major groove were characterized.

P-599

Large scale dynamics of immunoglobulin G

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Dynamical effects in diffusion-driven encounters between antibodies and foreign agents represent a problem of broad relevance to physics and biology. Based on a mechanical model parameterized directly upon results from single-molecule experiments, we investigate a typical antigen-antibody reaction chain. We demonstrate that the role of dynamics in the encounter process may be easily described within a simple, intuitive theoretical framework, that we formulate analytically. This enables us to show that the inner dynamics of antibody molecules results in a cooperative behavior of their individual sub-units. Our results constitute the first application of the DEER method, a thoroughly general integrated strategy to simulate large-scale functional dynamics of proteins, based on structural information extracted from single-molecule experiments.

P-600

Studies of the percolating network of hydration water by computer simulation

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The formation of a percolating hydrogen-bonded network by the hydration water and its disruption qualitatively change the properties of various physical and biological systems. For example, the appearance of a spanning water network coincides with the onset of biological activity of proteins with increasing hydration level. The thermal disintegration of the spanning network formed by hydration water of biomolecules in solution could essentially affect their structure and function. We have developed a method to study by computer simulations the 2D percolation transition of hydration water near various surfaces, including surfaces of biomolecules. The formation of a spanning water network is found to occur in a universal way in various systems: on infinite planar surfaces, on finite spherical surfaces, in protein powder, on the surface of biomolecules at low hydration levels and in solution. We observe strong fluctuations of the hydration water network close to the percolation threshold that could be important for protein function. We have found that the average number of water-water hydrogen bonds in the hydration water shell is about 2.2 at the threshold, independent of the system studied. Interestingly, this value does not change noticeably with temperature and surface properties. Hence, the average number of the hydrogen bonds in hydration water can be used for an easy location of the percolation transition of water in various biosystems.

P-601

Understanding ion selectivity through simulations with successively complex molecular models

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Monte Carlo simulations were performed with flexible and polarizable molecular models that can respond to the strong interactions with the ions, and whose parameters were fitted to ab initio calculations and gas-phase experimental data, to study aqueous solutions of Na+, K+ and Li+ confined within straight cylinders of various different radii, as described in [1]. Then, water molecules were placed at specific sites, to mimic the carboxyl oxygens in the selective filter of the KcsA potassium channel [2], and the ions were placed in three different locations, to get an estimate of their relative free energies with respect to their non-confined aqueous solutions. From the comparison of the free energies, evidence was found to support the proposal that the different hydration properties of the ions, mainly within the first hydration shell, produce selectivity.
[1] M. Carrillo-Tripp, H. Saint-Martin, and I. Ortega-Blake, *Phys. Rev. Lett.* **2004**, 93(16): Art. No. 168104
[2] D. A. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. MacKinnon, *Science* **1998**, 280: 69

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– Modelling Molecules –

P-602

Molecular dynamics study on the influence of TFE in mastoparan peptides folding and stability

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NMR and CD data have shown that mastoparan-like peptides from wasp venom have amphiphatic helical conformation in appropriate media. This feature seems to be fundamental for the bioactivities of these peptides. We studied the conformational properties of two peptides, Mastoparan-X (MX, PDB 1A13) and EMP-AF, by molecular dynamics using GROMACS package. The system was solvated in a SPC water and trifluoroethanol (30%TFE) cubic box with periodic boundary conditions. Temperature and pressure controlled with the Berendsen algorithm and PME corrections used for interactions far from the cutoff region (1.4 nm). We considered two starting conformations: the native one (NMR) obtained for TFE aq. soln. and another conformation with 5 helical residues. The results of simulations in water show the peptide in random conformation, irrespective of the starting conformation. It seems that the competition for hydrogen bonds with water unfold the native helix in some picoseconds (500 ps). In 30%TFE the native helix conformation is kept for 20 ns; however when starting with the other conformation, we found out that TFE induced 3 nearby residues into α-helix what is in accordance with experimental data. It seems that the clustering of TFE molecules around the peptide, mainly around the hydrophobic residues, could be the reason for the stabilization of helical conformation.

P-602-B

Structural characterisation of a complementary peptide inhibitor of the interleukin-1β

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It has been suggested that peptides coded for by sense and complementary strands of DNA interact specifically in a way similar to the Watson-Crick base pairs.
Two main theories have been proposed for the rational design of the antisequence to the investigated peptide. The first is the M-I theory which is based on the sense and complementary genetic code which argues that stability arises from specific side chain interaction. In other words, the genetic code and its complement are able to specify through space interactions between pairs of amino acid residues. Secondly, the MRT theory which argues that binding is due to inverted hydropathy when comparing sense amino acids with their antisense counterparts.
The mechanism of binding of the peptide QGEESND and its antisequence the VITFFSL is studied. The design of the antisequence was based on the M-I theory. The aim of this work is to use restrained MD simulations to study the structural and dynamical behaviour of this complex. The NMR restraints were taken from solution-state (DMSO) NMR experiments. Structural and energetic evidence based on enthalpic calculations support the â-strand conformation in a parallel manner. Entropy and the solvent interaction calculations are underway to determine the free energy of the entire system.

P-603

The role of DNA structure and dynamics in the recognition of BPV-1-E2 protein target sequences

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The papillomavirus E2 transcription and replication factors bind to the DNA consensus $ACCGN_4CGGT$ sequence (E2BS). In the E2/E2BS complex, the protein directly contacts the two half-sites ACCG.CGGT without forming any hydrogen bond with the four central base pairs (N_4). Nevertheless, the spacer sequence modulates the affinity. In order to investigate the role of the spacer and to better understand the recognition mechanism between E2 and E2BS, molecular dynamics simulations in explicit solvent were performed on *i)*three E2BS sequences containing different spacers conferring to the oligomers a very high, high or low affinity for E2 and *ii)* the complex of E2 with a high affinity sequence. The analysis of the trajectories focuses on the comparison of DNA dynamic and structural properties in the free and bound states. We show that E2 takes advantage of the pre-distorted structures of the free half-sites. In addition, our results strongly support that the best targets are distinguished from the low affinity sequences by an indirect readout mechanism in which the intrinsic plasticity of the non contacted spacer sequence, mainly manifest through the backbone motions, could allow to minimize the DNA deformation cost and to keep a low entropic penalty.

P-604

Platinated DNA modeled by AMBER

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The anti-tumor drug cisplatin (cis-diamminedichloroplatinum(II)) preferentially binds to GpG steps of DNA, forming N7,N7 intrastrands chelates. Our group has developped a force field parametrisation of the Pt-G junction which includes both the deformations and the electronic effect imposed by platinum on the bound guanines. However, recent NMR studies (Polak M. & al. Perkin Trans. 1, (19), pp 2895, 1999) have shown that the inductive effect of platinum does not stop at the guanine atoms but affects also the conformation of the sugar and of the 3' phosphate by stabilising sugars in North puckering and conformation ε^t for the phosphate. In the aim to improve the representation of DNA/platinum complexes, we have carried out molecular dynamic simulations with explicit solvent on two different DNA/platinum complexes: i) (CTCTG*G*TCTC)-d(GAGACCAGAG) where the guanines G* are chelated by the binuclear complex cis,cis-$\{Pt(NH_3)_2$-(m-pyrazolato)-$Pt(NH_3)_2\}^{3+}$. In this complex, platinum binding imposes moderate distorsions on the DNA that permit to separate electronic and steric effects of platinum binding on the sugar conformation ii) (GCCG*G*GTCGC)-d(GCGACCCGGC) where the guanines G* are chelated by cisplatin which produces a strong kink in the DNA. Models refined with unmodified Parm98 force field show discrepancies with NMR data for the ε conformation of the phophate 3' to the cross-link. This duplex is therefore a good test case for a parametrisation of the ε torsion angle.

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– Modelling Molecules –

P-605

3D-QSAR Analysis on DDPH derivatives for alpha-1–AR Antagonist Activity

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DDPH is as an antihypertensive agent in phase II trial now, but no binding structural information is available. Using Apex-3D program QSAR was studied on a series of DDPH derivatives The log ($1/IC_{50}$) values (pIC_{50}) were used to derive QSAR models. The result indicated 6 biophore sites which influence the bioactivity. The biophore model and 3D-QSAR equation can help us in understanding the receptor-ligand interaction, also in designing new compound with better potency.

P-606

Insights in the oxygenolysis mechanism of flavonoids by Quercetin 2,3-Dioxygenase

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Quercetin 2,3-Dioxygenase (noted 2,3QD) plays a key role in the degradation pathway of flavonoids by catalyzing their oxygenolysis in the presence of O_2. 2,3QD belongs to the cupin superfamily and is the only non-iron dioxygenase for which a crystal structure is characterized and the reactivity is unambiguously known to rely on a mononuclear copper center. Flavonoids are naturally occurring phenol derivatives that present several interesting biological activities, such as antiradical and anti-oxidative actions. In this study, quercetin flavonoid has been chosen as the substrate since it is often considered as the model compound for this chemical familly due to its wide spectra of biological activities.
On one hand, we have explored by means of Molecular Dynamics simulations the dynamical behaviours of this substrate embedded in the enzyme cavity and compared them to those of the substrate-free enzyme and, on the other hand, we have extensively investigated the electronic description of the substrate as well as the major trends related to the oxygenolysis reaction catalysed by the enzyme, such as reaction energies.
On the basis of these theoretical results, we show the role of aminoacids constituting the active site in the recognition and the activation processes of the substrate. Moreover, deep analysis of various structural and energetical characteristics allows us to postulate the existence of a dioxygen channel that should directly lead to the substrate embedded in the enzyme cavity.

P-608

Models to calculate biomolecular bond strength in flow chamber experiment

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Shear force flow chamber is commonly used to study biological interactions from cellular to molecular levels. Researchers often use a plane surface and microspheres (or cells) as counterpart carriers to perform molecular interaction. Goldman's mechanical analysis (1967) of a sphere deposited on a plane under a laminar shear flow is cited by many researchers to make further deduction about molecular mechanics. However, most previous work failed to consider both protein surface concentration and bonds' positional variation in their deduction to obtain the bond strength. An improved analysis was proposed in this work. With different simplifications of bonds' mechanical constitutive characteristics, two models to calculate the molecular bond strength were analyzed, which corresponded to a minimum and a maximum estimation, respectively. The minimum estimation model assumes bonds have super tensibility and the tension remains constant regardless the elongation of the bonds; the maximum one assumes bonds have little tensibility or they are tensible but the tension in the bonds decreases dramatically as they are stretched. The models were employed to quantify the bond strength between human IgG and goat anti-human IgG in our experiments, and the results showed that the antigen-antibody bond strength should be between 33.9 pN and 513 pN. This range is quite consistent with most other experimental results (such as by AFM).

P-607

Models to calculate biomolecular bond strength in flow chamber experiment

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Shear force flow chamber is a common used method to study biointeraction phenomenon from cellular to molecular levels. Researchers often use a plane surface and microspheres (or cells) as counterpart carriers to perform molecular interaction. Goldman's mechanical analysis (1967) of a sphere deposited on a plane under a laminar shear flow is a basis of many researchers to make further deductions about biomolecule mechanics. However, most previous work failed to consider both the surface protein concentration and the bonds' positional variation in their deduction of the bond strength. This work proposed an improved analysis. With different simplifications of bonds' mechanical constitutive characteristics, two models to estimate the molecular bond strength were analyzed, which corresponded to a minimum and a maximum limitation respectively. The minimum estimation model assumes bonds have super tensibility and the tension remains constant regardless the elongation of the bonds; the maximum one assumes bonds have little tensibility or they are tensible but the tension in the bonds decreases dramatically as they are stretched. The models were employed to value the bond strength between human IgG and goat anti-human IgG in our experiments, and the results showed that the antigen-antibody bond should have strength in the limitations between 33.9 pN and 513 pN. This range is much consistent with most other experimental results (such as by AFM).

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P-609

Binding mode of E-64 to Malaria and Chagas´ disease cysteine proteases

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Malaria and Chagas' disease continue to plague developing countries, threatening the lives of millions of people worldwide. Proteases from metabolic processes are potential targets for new chemotherapy and have led to important advances in the treatment of parasitic diseases. Cruzipain (CP1) and Cruzipain2 (CP2) from *T.cruz*i, and Falcipain-2 (FP2) from *P.falciparum* are essential parasite proteases, thus, the investigation their interactions with E-64, a potent class-specific cysteine-protease inhibitor, can elucidate features that compromise the binding affinity of E-64 and guide the design of new inhibitors. E-64 was docked to proteases based on the structure of Cathepsin-K (1ATK) and the adsorptive complexes were refined by energy minimization and submitted to 6.0ns Molecular Dynamics (MD) simulations in a cubic periodic boundary box, filled with ∼10K water molecules using GROMACS. Trajectories were analyzed to define the probable dynamics of the binding mode of E-64 to proteases. We verified that the ligand lies parallel to the active-center cleft remaining anchored by interactions to the binding pockets and short polar contacts with backbone atoms of the cleft. We verified that there are significant differences in protein-ligand surface contact area, hydrogen bonding prevalence E-64 positions in catalytic cleft that may explain the differences in binding affinity of E-64 and indicate the modifications to improve selectivity and specificity of ligand to each protease.

P-610

Mechanics of the B-DNA reflected in NMR by sequential distances and 31P chemical shift correlations

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By Nuclear Magnetic Resonance (NMR) and molecular dynamics (MD) in explicit solvent and using the two Parm98 and Charmm27 force fields, we have studied a DNA sequence related to the transcription factor Jun-Fos target, an heteroprotein that regulates a myriad of genes in a variety of tissues and cell types.
By carefully extracting the distances from NOE cross peaks and by assigning all the ^{31}P chemical shifts (δP), we highlighted strong cross-correlations between the sequential distances H2"-H6/8, H2'-H6/8 and H6/8-H6/8, themselves correlated to δP. In various MD, these correlations are retrieved, δP being substituted by the percentage of BII conformers. We conclude That the experimental correlations reflect and prove, in solution, the relationships between the sugar puckers, the BI/BII backbone conformations and the twist and roll helical parameters. Finally, a preliminary averaged structure extracted from constrained MD is presented the roll pattern and the resulting curvatureis discussed. In conclusion, due to these correlations, an accurate determination of the sequential distances using classical NOESY spectroscopy allows to check the coherency of NMR data and reinforces reliable information on the backbone dynamics, a fundamental element in determining DNA global structure.

P-611

Making a round dozen: construction of a 12-membered TRAP ring

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In bacteria, the expression of the trp operon can be controlled via an attenuation mechanism involving the protein TRAP which can bind to mRNA, stimulating the formation of terminating hairpin structures. The TRAP ring is formed from 11 identical TRAP proteins. By fusing 3 or 4 TRAP monomer genes we were able to express new subunits equal to 3 or 4 regular TRAP monomers, thus disallowing the formation of an 11-membered ring. Electron microscopy showed that the new proteins were, nevertheless, able to form ring structures. The crystal structures of both of the new TRAP rings have been solved, showing the new rings formed by these TRAP monomers are equivalent to rings consisting of 12 wild type TRAP monomers. The new rings are able to bind tryptophan at 12 sites and they closely resemble the standard TRAP ring but with significant deformation to accommodate the equivalent of an extra subunit. The new TRAP ring may prove useful in producing 2D arrays of metal quantum dots as well as helping to understand the tryptophan-binding mechanism of TRAP.

P-612

Morphology of detergent solubilized apolipoprotein B100

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Apolipoprotein B100 (apo B100), the major protein component of human plasma low density lipoprotein (LDL), is responsible for the secretion of triglyceride-rich lipoproteins and the receptor mediated endocytosis of LDL. It is one of the largest single-chain proteins with a molecular mass of about 550 kDa. Despite extensive efforts, sound knowledge on the detailed structural organisation of apo-B100 is still missing. In this study, we have solubilized apo-B100 from human LDL by the use of a non-ionic detergent. The lipid-free, detergent solubilized apo-B100 was stable for longer periods even at higher concentrations and revealed a secondary structure similar to that of apo-B100 in LDL, as shown by circular dichroism. The morphology of apo-B100 solubilized in detergent was assessed by negative stain electron microscopy. In comparison to native LDL the size of the solubilized apoB-100 was approximately twice as large. Finally, we used small-angle neutron scattering in combination with contrast variation to determine the size and shape of apoB-100 within the detergent complex. A three-dimensional low resolution structural model was reconstructed from the scattering data. The model revealed an overall length of about 60 nm for apo-B100 and suggested an arc-like domain structure with a distinct central pocket.

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Parallel superpleated beta-structure as a fold for amyloid fibrils

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In several neurodegenerative diseases, including Alzheimer's, Huntington's diseases and prion diseases, proteins that are normally soluble polymerize into amyloid fibrils that correlate with the disease state. Despite much effort, no high resolution structure has yet been determined for any amyloid fibril. Recently, we proposed a fold called the parallel superpleated β-structure as the structural basis for filaments of the yeast prion protein Ure2p and several other amyloids (1, 2). In this fold, β-strands zig-zag in a planar serpentine arrangement and serpentines stack axially, in register, generating parallel β -sheets with a small left-handed twist. The filament is stabilized by packing of apolar side-chains and by H-bonded ladders of polar residues. This molecular model emerged after a survey of experimental data on Ure2p. We have also found that a shorter three-stranded serpentine design is consistent with experimental data on human amylin protofilaments whereby two or three protofilaments coil into fibrils (2). We further envisage that amyloid fibrils of huntingtin, Sup35p, α-synuclein have similar architectures (1). These observations have potential for the identification of amyloidogenic protein sequences and the discovery of therapeutic agents for the treatment of amyloid diseases.
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P-614

Proton transfer through peptide bond in enol-keto transformation

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While side chains provide physiological functions to proteins, peptide bonds constitute their backbone and are formed by chemically stable keto forms [-CO-NH-]. Recent experiments suggest, however, that the peptide bond can assume a metastable structure in enol form [-C(OH)=N-] in basic environments and an enol-keto transformation might cause proton transfer. In this process, a proton must pass through the peptide covalent bond and this differs from conventional proton transfer though hydrogen bonds. Yet, to date no detailed calculations able to unravel this point, not directly accessible to experimental probes, have been reported.
In order to investigate the two possible reaction paths involved, we first constructed ideal and realistic models extracted from the real system, then performed static total-energy calculations based on density functional theory and dynamical Car-Parrinello simulations. In these two reaction paths, we identify transition states characterized by 4-membered rings in the case of direct transfer, or 5-, 7-membered rings in the case of indirect transfer; the most probable (i.e. lowest activation barrier) pathway is not a direct proton transfer, but an indirect mechanism involving two protons. This is partly due to the fact that 5-, 7-membered rings are easier realized in physiological conditions.

P-615

pH-coupled molecular dynamics simulations of proteins

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pH-dependent conformational transitions play important roles in many physiologically relevant biological processes, such as the fibril formation of amyloidogenic proteins, infectivity cycles of viruses such as polio, influenza and HIV, and the activation of bacterial toxins. Here, we present a state-of-the-art constant-pH molecular simulation technique (CPHMD) based on the lambda dynamics and Generalized Born solvation methods. The accuracy and efficiency of the CPHMD technique are demonstrated through pKa calculations of ovomucoid turkey third domain and ribonuclease A, where the sign of pKa shifts is reproduced for all residues but one. The tautomeric titration sites for histidines obtained from the simulations are in agreement with experiment. The average absolute errors of the computed pKa's for the carboxyl, histidine, and amino groups are 1.4, 0.6, and 0.6 pKa units, respectively. The CPHMD technique is also applied to explore the pH-dependent helical propensity of Alzheimer's beta peptide (1-28) in water. Analysis of 45-ns pH-coupled molecular dynamics trajectories at room temperature shows the least helical content at pH 6 and 7, in general agreement with experiments carried out in the aqueous solution of TFE. Salt bridges and hydrophobic interactions are found to be responsible for the disruption of the backbone hydrogen bonds. These applications demonstrate the potential of CPHMD simulations in revealing pH-dependent local side-chain and global conformational changes in proteins.

P-616

Recent advances and applications of the Fragment Molecular Orbital method (FMO) in biomolecular computation

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Computation of the electronic state of large molecules has recently become possible by the Fragment Molecular Orbital (FMO) method [1]. FMO is a highly parallelizable and scalable method, and is therefore suitable for large scale ab initio MO calculations of biological molecules.
The FMO method has been implemented into the ABINIT-MP [2], and GAMESS [3] programs. The ABINIT-MP program has been merged with a molecular modeling package PEACH to implement the FMO-MD method [4, 5].
In this poster, recent advances in the FMO methodology are reviewed. Then, an FMO computation of the Catabolite Repressor Protein-DNA complex is presented. Recent development of FMO-MD method will be also presented. The applicability of FMO in biomolecular computation and simulation will be discussed.
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P-617

A genetic algorithm for homology model optimization using residual dipolar coupling and SAXS data

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We try to elucidate the molecular bases of the S1 ribosomal protein action, which is essential to the recognition of the mRNA translation initiation region by the ribosome. One way to approach this problem would be to resolve the structure of S1 RNA binding region and of various S1/RNA complexes. The size of the objects makes this impossible by classical NMR techniques. On the other hand, an X-ray study would require the tedious optimization of crystallization conditions for each complex. However, S1 is formed of repetitions of a conserved domain, for which similar structures are known. We tried to develop a method to refine homology models of the modules and to position them in the protein.
We examined whether it is possible to fulfil this objective using only NMR Residual Dipolar Coupling and SAXS data, by the means of a simple adaptative genetic algorithm To test our procedure, we built homology models of S1 and KH modules of *M. tuberculosis* NusA protein using the X-ray coordinates of *T. maritima* NusA. We then tried to refine each module and to determine their relative position in the protein by using "experimental" data issued from the *Mycobacterium* NusA X-ray structure. Our results show that our genetic algorithm works well, that RDC alone are not sufficient to refine module model but that SAXS adjunction improves the process and that it seems possible, using this method, to reconstitute the architecture of bi-modular proteins.

P-618

Comparative modeling of the poplar plasma-membrane aquaporin PttPIP2.5

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Unlike animals where only two aquaporin subfamilies have been described so far, plants have four distinct subfamilies of aquaporins. The function of majority of those has never been experimentally explained. Efficiency of those that were proved to be water specific channels is not equal. Aquaporins located to plasma-membranes were measured as the most effective plant water channels when expressed in oocytes of *Xenopus laevis*, where few of them caused as strong Pf values as animal aquaporins.
PttPIP2.5 is the strongest measured plant aqauporin in the oocytes-system so far. As the sequence analysis showed some particular features of this protein, homology-modeling studies were employed to shed light on the structural basis for the high efficiency in water transport. Amino acids forming the water channel of the modeled structure are compared to the amino acids of the template (bovine AQP1) based on a structural alignment. The physical-chemical properties of these amino acids are described through the Sting Database, highlighting the differences that may be responsible for the functional specificities among the aquaporines.

P-619

Protein Conformational changes: Experiments versus simulations

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In this study we analysed by Targeted Molecular Dynamics (TMD), the activation conformational change of Chymotrypsin and 4 of its mutants.
The activation of chymotypsinogen into chymotrypsin happens via the cleavage of the R15-I16 bond and the subsequent rotation of residue I16 from the solvent into the interior of the protein. The transition can be induced by basic to neutral pH change. The kinetics of the activation process can be followed by FSF experiments while the structural features of the transition can be explored by *in silico* simulations.
The impact of mutations on the conformational transition of rat-Δα-chymotrypsin, and the different activation pathways generated have been investigated in terms of structure descriptors such as the behaviour of M192, G193 and the I16-D194 salt-bridge and by estimating the activation enthalpy and entropy values. In addition the changes in the behaviour of the Tryptophan residues and their environment were analysed.
Despite the limited extent of the conformational change, the calculated pathways were observed to be a sequence of many steps resulting in free energy trajectories consisting of multiple transition states. When multiple simulations were carried out on each enzyme TMD produced also pathways with an increase of free energy.

P-620

Predicting the structures of electron transfer complexes of nitrous oxide reductase

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Electron transfer (ET) complexes are difficult to crystallise but structures of many uncomplexed ET proteins are known. This, together with the fact that, upon complex formation, these proteins are thought to undergo only small structural rearrangements, makes ET complexes interesting objects for protein-protein docking experiments. In our docking protocol, we used CHARMM force fields combined with EEF and ACE solvation modes to score the candidate ET complexes generated with FTDOCK. The results show that the ACE solvation model improves the accuracy of scoring. However, satisfactory results were achieved only when the candidate complexes were first filtered using a restraint based on the distance between the electron donating and accepting groups. After testing our protocol with a known structure of an ET complex, we used the protocol to predict the interaction of nitrous oxide reductase (N_2OR) with its electron donors cytochrome c_{550} and pseudoazurin. The results suggest that both donors utilise the same binding site but have multiple, rotationally related ways to form a complex that enables rapid electron transfer to the Cu_A centre of N_2OR. In all complexes, the actual protein-protein interaction site consists of mainly hydrophobic surface residues. The dipole moments of cytochrome c_{550} and pseudoazurin and the charged surface amino acids of N_2OR also contribute to the binding process.

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P-621

New Force Field Parameters for MD Simulations of the Ca-Binding Sites in Annexin-Membrane complexes

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For accurate classical molecular dynamics (MD) simulations of the calcium mediated bound complexes of annexin and membrane we have developed new force-field parameters describing correctly the interaction of the Ca ion with its environment. We have used quantum chemical calculations to investigate the potential energy surface experienced by the Ca ion within the three different binding sites found in domain 1 of annexin V (ANX V/1). Based on these calculations we were able to quantify the charge polarization of atoms within the binding sites, and to determine the geometry and force constants of harmonic restraints between Ca ion and its coordinating oxygen atoms. Harmonic restraints were introduced to compensate for the deviations between the quantum mechanical potential energy surface and that of the classical force field. Our analysis has shown that using the refined force field for the Ca binding sites enables long-time MD simulations which conserve the initial structure of ANX V/1 significantly better than MD simulations using the standard force field.

P-622

The development of fast free energy calculations methods and their application to drug design

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The computational prediction of physical and chemical properties of biological systems is becoming increasingly important in life science. In drug design, the search for new inhibitors of a particular target can greatly benefit from theoretical predictions, particularly of the free energy of binding. Statistical mechanics provides a route to the calculation of this quantity(1). Free energy calculations on protein-inhibitors complexes are usually computationally expensive, which has prompted the development of many approximate free energy methods(2). In this work, we are aiming at developing a free energy method that is more efficient than conventional free energy simulations while keeping a rigorous statistical mechanics framework. We achieve this by combining recently developed methods that yield enhanced sampling of phase space(3) and by adopting a continuum electrostatics approach that simplifies the free energy landscape(4). The application of these methods to a set of inhibitors of the influenza enzyme Neuramidinase will be discussed.

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P-623

Bioactive form of glyphosate: DFT based molecular electrostatic potentials and docking studies

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In structure based ligand design, identification of the bioactive form is an important task. Generally, for this, choosing conformers with energy cut of values ranging from 3-5 kcal/mol is not a proper way. Therefore, the most appropriate method is to select them based on their scoring function obtained from rigid docking of conformers which in turn are obtained from ab initio QM studies and couple them with the electrostatic potentials. Such an approach has been applied by us to determine the bioactive form of an important herbicidal ligand *viz* glyphosate. As glyphosate contains four different protonation sites, it exhibits five different ionic forms (+1, 0, -1, -2 and –3) in solutions depending upon the pH values. Therefore, it is necessary to identify the best form of the ligand for its proper biological function. In this regard, computations (scoring energy, MESP and conformational analysis) have been carried out on the various ionic forms of the ligand. Our studies reveal that the –2 form is the bioactive one. This prediction is in very good agreement with the results of X-ray crystallographic studies. The method and the other results obtained will be presented in detail in the symposium.

P-624

Detailed microscopic study of the full ZipA-FtsZ interface

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Protein-protein interaction networks are very important for a wide range of biological processes. Alanine scanning mutagenesis has allowed the discovery of the hot spots [1] - energetically important residues for complex formation. To a better understanding of the binding determinants of the complex formed between the FtsZ fragment and ZipA we extended the alanine scanning mutagenesis study to all interfacial residues of this complex. As a result, we present new mutations that allowed the discovery of residues for which the binding free energy differences upon alanine mutation are higher than 2.0 kcal/mol. We also observed the formation of a hydrophobic pocket with a high warm spot spatial complementarity between FtsZ and ZipA.
Small molecules could be designed to bind to these amino acid residues hindering the binding of FtsZ to ZipA. Hence, these mutational data can be used to design new drugs to control more efficiently bacterial infections.
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P-625

GMPC model and theory of helix-coil transition in one- and double stranded biopolymers

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The Hamiltonian of the Generalized Model of Polypeptide Chain (GMPC) is introduced to describe the one- and double stranded system. The Hamiltonian uses only pure molecular microscopic parameters (the energy of hydrogen bond (HB) formation, reduced partition function of repeated unit, the number of repeated units fixed by one HB, the energies of interaction between the repeated units and the solvent molecules). The transfer-matrix approach has been used for observable parameters estimation. We succeed to describe the influence of solvents, both competing and non-competing for intra-molecular HB formation. We have considered stacking and hydrogen bonding simultaneously, taking into account regular and random heterogeneity. The influence of solvent-macromolecule interaction energy on the melting temperature and melting interval has been considered. It has been obtained that stacking interaction on the background of HB increases stability and decreases cooperativity of melting in Nucleic Acids. For random heteropolymeric system we calculate transition temperature and interval of melting. For regular heteropolymeric system we considered both roles of stacking and HB heterogeneity on stability and cooperativity.

P-626

Protein-oligosaccharide interaction mechanisms in chitinases engineered towards neolectins

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Specific protein-carbohydrate interactions are fundamental to many biomolecular recognition events. The study concentrates on fungal 42kDa chitinase from *Trichoderma harzianum*, a naturally chitin (polymer of N-acetylglucosamine residues) degrading enzyme. It has shown activity also on synthetically modified α-1,3-fucosylated and β-1,4-galactosylated, more animal type of chito-oligosaccharides. The structure of chitinase (α/β-barrel fold) with the substrate binding cleft formed by limited number of loops provides an excellent platform for directed evolution studies. In this study our aim is to obtain atomic level understanding of the factors determining the interactions between an oligosaccharide and a protein by using molecular dynamic simulations. The results from modeling are compared with the experimental data (mutagenesis, mass spectroscopy and nuclear magnetic resonance). The computational studies with the experimental work aim at development of neolectins, i.e. proteins selectively binding to given, medically important, oligosaccharide structures, achieved by first deactivating and then engineering fungal chitinases towards the desired specificity and affinity.

P-627

Spatial structure and conformational peculiarities of the allatostatin family neuropeptide

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In this report the spatial structure and conformational possibilities of insect allatostatin IV (Asp-Arg-Leu-Tyr-Ser-Phe-Gly-Leu-NH_2), the peptide that strongly stimulates juvenile hormone biosynthesis by the "corpora allata" were investigated by the method of theoretical conformational analysis and molecular dynamics simulation in water solution. Calculations are being carried out on the basis that stable conformations of peptide in water solution correspond to local minima of a function which is the sum of the potential energy for all intra-peptide interactions and the free energy for all interactions involving the solvent. The stable conformational states corresponded to the minimal values of this energy of the peptide molecule have been established. The calculations result indicates that allatostatin IV neuropeptide is relatively flexible molecule which can exist in several conformations. The lowest energy conformer namely the global conformer has a folded N-terminal part of the molecule including into the sequence Tyr4-Gly7 the integrity of which is needed for high potency. Interactions between Tyr4 and Gly7, and between Phe6 and Leu8 are characteristic for all low-energy conformers. According to the calculation result Arg2, the electrically charged amino acid within C-terminus makes an important contribution towards the electrostatic interactions to the stabilization of the peptide molecule in water solution.

P-628

Mutation effects on structure and dynamics of N-terminus of tyrosine hydroxylase

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The molecular mechanics and molecular dynamics computational methods were used to predict the low-energy conformational states of the amino acids sequence Met1-Val60 from the N-terminal regulatory domain of the tyrosine hydroxylase (TH), the key enzyme of catecholamine biosynthesis. The effect of the amino acids substitution on the behavior of the peptide segment in water solution has been studied. The following point mutations in the primary structure of the Met1-Val60 peptide segment were used in computational experiments: (i) Arg37-Arg38 replaced by Gly37-Gly38; (ii) Arg37-Arg38 replaced by Glu37-Glu38; (iii) Ser40 replaced by Asp40, and (iv) Ser40 replaced by Ala 40. Calculation results indicate that the stretch of amino acids Ile29-Leu41 intervening between the two helices capable forms reversible bend on the Pro32-Ile35 and Phe34-Arg37 segments. Two alpha-helices including residues Arg16-Ile29 and Leu41-Val60 are parallel to one another in calculated lowest energy state of the peptide backbone. It was established that the mobility of the Ser40 side chain restricted in comparison to Ser31 and so may be sensitive to enzyme phosphorylation state and to dopamine binding. Significant differences are observed for the Arg37→Gly37 mutant segment indicated the most important role of the Arg37 in the regulation TH activity. Our results are useful tool to determine solution structure for select part of the enzyme, especially in the absence of crystallographic data.

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P-629

Analytic formulation of the stochastic energization-relaxation channel model of active transport

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The active transport of ions builds up and maintains electrochemical potential gradients across biological cell membranes. It is performed by ion pumps: membrane proteins fueled by energy-releasing processes. The stochastic energization-relaxation channel (SERC) model of active transport describes the pump as a multi-ion channel with two conformations, termed energized and relaxed, of specific free energy profiles. Here we present a new, analytic formulation of the SERC model, based on differentiation formulae from the theory of stochastic differential equations. The energization/relaxation switches are described in terms of a Markovian dichotomous noise. Upon averaging the kinetic equations over the noise, the mean ion flux per pump molecule is obtained as a function of time. Its asymptotic value, the stationary ion flux generated by one transport protein, is a measurable quantity, found to be in qualitative agreement with experimental results on bacteriorhodopsin, a light-driven proton pump.

P-630

Reorganization and conformational changes in the reduction of tetraheme cytochromes

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Redox changes in proteins are usually accompanied by conformational changes. In this work, we studied cytochrome c_3, in the fully oxidized and reduced states, using MD Simulations. These proteins are well characterized experimentally, structural and functionally, making them suitable for studies with the purpose of understanding the redox reorganization phenomena.
As shown by our results, doing only one simulation for each state can lead to unreliable conclusions. So, to guarantee the existence of enough statistics we performed ten 4ns simulations for each redox state. All simulations were performed with GROMOS96 force field (with a new set of charges for both states calculated with GAUSSIAN98 and RESP fitting) in the GROMACS 3.1.4 package.
The results obtained with this statistically robust methodology show the existence of subtle conformational changes that can be correlated with the experimental structural data, showing that these techniques, once properly used, are indeed adequate for studying this type of process. Additionally, we study several other molecular aspects: the variation in the number of H-bonds, the solvent accessible surface and bound water molecules. The simultaneous effects of protonation and reduction were also investigated, by analyzing the two most probable groups for this to happen, propionates D from hemes I and IV.

P-631

Studies of de novo designed alpha-helical coiled-coil peptides

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The α-helical coiled coil structures are recognized as one of nature's favourite ways of creating an oligomerization motif. Proteins with the coiled coil pattern form two- to five-stranded oligomers depending on their sequences. We focus our studies on the establishment of the interactions that favour formation of the coiled coils with the maximal number of strands. A previous designed alpha forming peptide (αFFP) forms α-helical fibrils with 2.5 nm diameter in agreement with both four- and five-stranded coiled coils. In this work, several αFFPs were fused with small non-coiled coil peptides. CD spectroscopy, electron microscopy, sedimentation-diffusion and small angle X-ray scattering experiments show that addition of these non-coiled coil fragments blocks propagation of the coiled coil structure along the axial direction. The new peptides self-assembly into five subunit oligomers. The obtained soluble oligomers open possibility for the determination of their atomic structure by X-ray crystallography and NMR and this, in its turn, can provide insight into the atomic structure of αFFP nanofibrils.

P-632

Molecular Dynamics Simulations of N3´ to P5´ Phosphoramidate Modified DNA Quadruplex in solution

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Molecular Dynamics Simulations of N3' → P5' Phosphoramidate Modified DNA Quadruplex in solution
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The structural impact on modified tetraplex was investigated by carrying out molecular dynamics simulation on a deoxyribose quadruplex and its phosphoramidate (replacing O3' by NH) analog using particle mesh Ewald electrostatics and the Cornell et al force field. The NH group can adopt different orientation (characterized by the values of the C4'-C3'-N3'-H angle in the range 40° to 80° (tetpiNa) and - 40° to - 80° (tetpoNa). The exposure of the N-H to the water solvent plays a critical role on the observed conformational behavior of quadruplex during molecular dynamics simulations for tetpiNa and tetpoNa. The multiple simulations were performed for both the cases. The simulations carried out are consistent with the observed X-ray structure for the unmodified quadruplexes and depend on the initial location of the N-H hydrogen. Due to the presence of phosphoramidate the conformational change in the quadruplex structure, the variation of the backbone torsion angles and helicoidal parameters have been examined. The effect of hydration and motion of ions around quadruplex structure is also discussed.

Posters
– Modelling Molecules –

P-633

Protein-protein interactions: Modelling the hepatitis C virus ion channel p7

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The p7 protein is a small membrane polypeptide encoded by the hepatitis C virus and consists of two transmembrane á-helices, TM1/TM2. When inserted in artificial lipid bilayers, it assembles into cation selective ion channels that are blocked by long-alkyl-chain iminosugar derivatives. However, no structural information is available to explain the compounds' action. With this in mind, the results of modelling studies on the p7 structure are presented. Our modelling work proceeds in a hierarchical manner in agreement with the two-stage model of membrane protein folding. First, the length of TM1 and TM2 is estimated by employing different secondary structure prediction algorithms. Subsequently, a configurational global search protocol based on simulated annealing and restrained molecular dynamics simulations is used as well as protein-protein docking approaches in order to investigate the packing of TM1/TM2, yielding a number of possible TM1/TM2 helical dimers. The stability of these dimers is then evaluated with molecular dynamics simulations in a fully solvated lipid bilayer. Finally, full p7 oligomeric bundles are generated and their biological implication as well as their consistency with the existing biochemical data is discussed.

P-634

Introduction of morphological and dynamical constraints in the modeling of protein super-complexes

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Atomic Force Microscopy (AFM) is based on scanning an extremely sharp tip across a surface, for instance a single molecule of protein on mica. AFM produces morphological envelops of molecules with a sub-nanometer resolution. These envelopes can be used to constrain the computation of large macromolecules or super-complexes. We illustrate the concept of this approach by showing high resolution imaging of single molecules of an antibody-antigen complex. High-resolution images were obtained using the intermittent contact mode of AFM on mica. Single molecule images of antibodies are consistent in size and volume with atomic-resolution X-ray crystallographic structures. This preliminary works pave the way for practical applications of AFM in molecular modeling. In addition, AFM technique allows the measurement of the interaction force between a macromolecule and a ligand attached at a tip mounted on a flexible cantilever. Molecular forces are specific physico-chemical properties of a single macromolecular complex. Combining measurements of topography with force provides key elements in the modeling of large super-complexes.

P-635

Chelation of Cu(I) and Hg(II) by metallochaperones: a comparative study by molecular dynamics

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Heavy metals can be toxic when present in inappropriate amounts or oxidation states in the cell. Some of them are nonetheless essential for protein structure and function. Among them, copper is used as a cofactor in several enzymes involved in redox chemistry. Organisms have developed systems for selectively transporting and sequestering copper in the cell.
In yeast, copper is imported as a Cu(I) ion and transferred to a soluble protein, Atx1, that conveys Cu(I) to a specific partner protein, Ccc2, a P-type ATPase. Similarly, in bacteria, mercury is bound by the protein MerP and transported to the mercury detoxification system. Atx1 and MerP belong to a class of metal-binding proteins, called metallochaperones. These proteins are small, highly homologous and all share the metal-binding sequence MXCXXC.
In order to get insight into the structural and dynamical properties of the metallochaperones, we have performed molecular dynamics simulations using potential energy functions developed in the lab. We have studied Atx1 and its bacteria and human homologues, MerP and Hah1 respectively, and the metal-binding domains of their ATPase partners. We have compared the structure and dynamics of these proteins in their apo, Cu(I)- and Hg(II)-forms.

P-636

Inferential structure determination

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Macromolecular structures calculated from nuclear magnetic resonance data are not fully determined by experimental data, but depend on subjective choices in data treatment and parameter settings. This makes it difficult to objectively judge the precision of the structures. We use Bayesian inference to derive a probability distribution that represents the unknown structure and its precision. This probability distribution also determines additional unknowns, such as theory parameters not being accessibly by experiment, that previously had to be chosen empirically. We implement this approach using Markov Chain Monte Carlo techniques. Our method provides an objective figure of merit, improves structural quality and minimizes human bias.

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Three-dimensional structure of ancymidol by X-ray crystallography and nuclear magnetic resonance

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Plant growth regulators (PGRs) have been important components in agricultural production even prior to the identification of plant hormones. The Ancymidol (alpha-cyclopropyl-(p-methoxyphenyl)-5-pyrimidinemethanol) is a synthetic vegetal growth regulator, that is part of the heterocyclical compound group that contains nitrogen and it inhibits the synthesis of the giberelines. The structure of this molecule was determined by X-Ray Crystallography and Nuclear Magnetic Resonance. The X-Ray data for the crystals of the title compound were collected by graphite-monochromatized Mo Kalpha radiation at 293 K. The structure was solved by direct methods and refined by full-matrix least-squares with anisotropic temperature factors for the non-hydrogen atoms. The spectrums of Nuclear Magnetic Resonance were made using a Bruker Spectrometer of 500 MHz with a field of 11,7 T. In the obtained structure three cycles were observed: pyrimidine, methoxyphenyl and cyclopropyl. The comparison of these two structures showed similar results. These agree in the rings planarity and their spatial distribution.
Acknowledgments: R. C. and O. O. thanks to Universidad Javeriana for financial support.

P-638

Computational Characterization of two family 1 beta-glucosidases

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Computational methods have been used to characterize substrate specificity and investigate the mechanism in two family 1 _-glucosidases, Glu1 and Dhr1. Glu1 hydrolyzes DIMBOA-glc, its natural substrate, and is inhibited by dhurrin. Dhr1 hydrolyzes dhurrin, its natural substrate, and is inhibited by DIMBOA-glc. Experimental data do not provide a complete understanding as to why Glu1 is inhibited by dhurrin. Molecular dynamics (MD) simulations have been performed on Glu1, Glu1 with DIMBOA-glc, Glu1 with dhurrin, and a Glu1/Dhr1 chimera with dhurrin. These simulations indicate that dhurrin inhibition is caused by distortion of the interactions between the aglycone moiety of the inhibitor and W378, an amino acid in the active site. Motion of W378 obstructs the active site and alters the orientation of the glucose moiety relative to the catalytic glutamates. We do not observe extensive W378 motion in the Glu1 with DIMBOA-glc nor Glu1 simulations. Our MD results also have implications on the current view of the mechanism of retaining glucosidases. A distance of <5.5Å between the catalytic glutamates is generally accepted. Our results indicate that the distance between the glutamates is closer to 3.0Å, which support an in-plane protonation hypothesis. Also, one of the glutamates, the acid/base catalyst, must have a perturbed pKa value so that it is protonated in the first step of the mechanism. Analysis of the pKa values of the X-ray Glu1 structure and the structures after dynamics shows that an expected pKa is only obtained after MD.

P-639

A 3D model of the nitrogenase/FeSII complex from Azotobacter vinelandii

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Nitrogenase complexes catalyse the ATP dependent reduction of dinitrogen to ammonia during a process called nitrogen fixation. Understanding the regulation of the nitrogen fixation process is of major importance in itself and in exploring the benefits of economically important plants.
To protect nitrogenase from inactivation by oxygen some diazotrophs developed a variety of mechanisms. *Azotobacter* species and *Gluconacetobacter diazotrophicus* use two major mechanisms that contribute to this protection, increased respiration and conformational protection. The conformational protection involves the association of the FeSII protein , but until now the structural information about this protein-protein interaction was not specified.
We derived a homology model of the 3D structure of the FeSII from *A. vinelandii* protein by comparative modeling technique. Analysing the resolved x-ray structure from A. vinelandii nitrogenase with computational methods, we were able to understand the interaction between the FeSII protein and the nitrogenase complex from *A. vinelandii*.
The proposed interaction between FeSII protein and nitrogenase prevents the dissociation of the Fe protein from the MoFe protein. The electron transfer between the proteins is inhibited protecting the nitrogenase from inactivation.

P-640

Using a mesoscopic model to unravel hairpin folding dynamics: Which are the relevant factors?

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To gain a deeper insight into cellular processes such as transcription and translation, one needs to uncover the mechanisms controlling the configurational changes of nucleic acids. As a step toward this aim, we present here a novel mesoscopic-level computational model that provides a {\it new window} into nucleic acid dynamics. We validate the model by studying DNA hairpins, single-stranded molecules with two complementary segments (``stems'') linked by a non-complementary ``loop.'' Our model reproduces experimental observations and enables us to monitor the configurational dynamics of hairpins, providing clear evidence of a ``zipping'' process in the closing toward the
native configuration. Our model allows us to demonstrate that there is a preferred zipping pathway for folding which is both the most frequent and the fastest way for the hairpin to fold.
Interestingly, our model allows for the tunning of the hydrogen-bonding and stacking interactions , enabling us to asses which are the relevant interactions controlling the folding process. Our simulations clearly show that stacking interactions are crucial for the hairpin to fold via a zipping process starting close to the loop. In the absence of stacking between bases, the closing starts from the strongest bonds following no particular path to fold into the native conformation.

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P-641

The alpha helix dipole: screened out?

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Aligned alpha-helix peptide dipoles sum to a 'macroscopic' dipole parallel to the helix axis that has been implicated in protein folding and function. However in aqueous solution, the dipole is counteracted by an electrostatic reaction field generated by the solvent and the strength of the helix dipole may reduce drastically from its value in vacuum. Here, using atomic-detail helix models and Poisson-Boltzmann continuum electrostatics calculations, the net effective dipole moment, μ_{eff} is calculated. Some at first sight surprising results are found. Whereas in vacuum μ_{eff} increases with helix length, the opposite is found to be the case for transmembrane helices. In soluble proteins, μ_{eff} is found to vary strongly with the orientation and position of the helix relative to the aqueous medium. Contrary to what the structural biology community commonly assumes and teaches that helices possess significant whole-helix dipole moments, we show that the helix dipole depends largely on the helix environment. A set of rules is established to estimate of the strength of μ_{eff} from graphical inspection of protein structures.

P-642

Complex-type-dependent scoring functions in protein-protein docking

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Scoring functions have been the essential part in the docking procedure, which help screen out the prospective candidates from the decoys pool to make binding mode prediction really meaningful. Here, we designed four combinatorial complex-type-dependent scoring functions through categorizing 64 target complexes into four groups. 30,000 docked decoys were generated by FTDock for each case of all targets. Top 2000 structures with least RMSD of each bound docking results of the 58 targets were utilized to fit the weights of the scoring functions. Unbound docking results of every target were used to test the discriminative capacities of various combinatorial scoring functions. Evaluation on the functions demonstrated overall outstanding performance for all cases, and especially exceptionally encouraging for the protease/inhibitor and antigen/antibody classes. The success rates for protease/inhibitor, antigen/antibody, enzyme/inhibitor and other types were 89%, 70%, 75% and 64%, respectively. These results validated our divide-and-conquer strategy on scoring functions development, which might hopefully shed some light on the future study on the protein-protein interactions.

P-643

Insights into the GB1 fold domain swapping mechanism by molecular dynamics simulations

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We used Designer, a fully automatic procedure for predicting the amino acid sequences compatible with a given target structure based on the CHARMM package, to investigate how specific mutations in the B1 domain of protein G, known to trigger dimerization, would behave when forced to fit in the wild type fold.
From the above work, we proposed a new mechanism that could possibly be the cause behind dimerization, differently from the initially proposed model of clashes between V39 and A34F (Byeon et al., 2003). In our model, A34F would clash with W43 by forcing it to adopt a less favorable rotamer, in addition to a more unfavorable long range interaction between A34F and V54.
This model, together with several analyses done for Molecular Dynamics (MD) simulations of 5 ns for the wild type structure and the dimeric sequence in the wild type fold at 300 K, showed that the second hairpin undergoes considerable conformational rearrangements compared to the control simulation, which could be an indication of the intrinsic fluctuations created by the mutations that eventually lead to the swapping phenomenon.
Reference: Byeon IJ, Louis JM and Gronenborn AM. J. Mol. Biol. (2003) *333*, 141-152.

P-644

Resorcinolic lipids - disturb or stabilize biological membranes? Molecular dynamics simulations

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Among many biological activities exerted by resorcinolic lipids very specific is their influence on the biological membranes.
Experiments have shown that resorcinolic lipids have a dual effect on the cell membrane and also on the model membrane - liposome. Preincorporated, they occur stabilizing and ordering effect on the phospholipid membrane. The size of modified liposomes by resorcinols is kept unchanged much longer and marker is released much slower. If resorcinols are incorporated into liposomes suspension the instantaneous marker release and erythrocyte hemolysis is observed.
Nowadays computer simulations methods are useful tool to understand the interaction of membranes and drugs and their influence on the disorder of the bilayer structure.
We present the molecular dynamics simulations of phospholipid membran with various homologs of resorcinolic lipids at the atomistic level and thereafter applying coarse grained model.
A data from the simulations sustain experimental results. We observe increase of the order parameter and diminish area per lipid what suggests the growth of membrane stiffness when they are preincorporated. And also diffusion of water through the membrane is higher when resorcinol molecules are incorporated into the external leaflet from the water solution.

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P-645

On the percolation transition of water in hydrated lysozyme

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The formation of a spanning hydrogen-bonded network of hydration water on protein surfaces via a percolation transition is closely connected with the onset of their biological activity [1]. We performed the first computer simulation study of the percolation transition of water in a model lysozyme powder and on the surface of a single lysozyme molecule [2]. The formation of an infinite water network in the protein powder occurs as a 2D percolation transition at a critical hydration level, which is close to the values observed experimentally [1]. The formation of a spanning 2D water network on a single lysozyme molecule also occurs via a 2D percolation transition and corresponds to the first appearance of a "monolayer" water coverage, which restores the full dynamics of lysozyme. The radius of gyration of the spanning cluster of hydration water always exceeds the radius of gyration of the protein. Any spanning cluster envelops essentially more than half of the surface area. Our simulations furnish a microscopic picture for the understanding of the specific values of the experimentally observed hydration levels, where different steps of increasing mobility in the hydrated lysozyme system are observed.

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(2) A. Oleinikova, N. Smolin, I. Brovchenko, A. Geiger, R. Winter, J. Phys. Chem. B 109, 1988 (2005)

P-646

Quantum mechanisms of long-distance communication between nucleotides in DNA

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We report an appearance of electron singlet (S) – triplet (T) state entanglement on approaching initially separated in space and oriented in opposite, 3'-5' and 5'-3', directions cytidine (C) and guanosine (G) nucleotides, according to Watson-Crick (W-C) pairing rule. The results are based on CI UHF MD quantum chemistry computations (2 10^6 configurations, 6-311G** basis set, T = 310°K) for T and S paths over a distance of 6.0 - 2.4 Å between nucleotide planes in a bath of 32 water molecules. The computations show that the difference in the total energy ΔE^{tot} between T and S path does not exceed 3.5 kcal/mol over a distance (Δr) of 6.0 - 4.0 Å; in a closer region, 3.85 - 3.2 Å, dramatic oscillations in E^{tot} occur, revealing, when imposed, multiple intersections between T and S paths. On reducing Δr, electron spins, located on C and G aromatic planes, alternately change their orientation, showing T-S spin entanglement. The effect has a diabatic nature, suggesting that the total wave function, due to presence of non-adiabatic potential, breaks its spatial and, thus, spin symmetry, becoming a complex variable with a non-vanishing in time phase. Strong coupling with diabatic behavior comes from the fact that in our computations we observed highly pronounced vibrations of C and G subsystems on approaching each other.

P-647

Quantum mechanisms of long-distance communication between nycleotides in DNA

V. E. Stefanov, A. A. Tulub, Y. I. Vaskov
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P-648

BioSimGrid: towards a worldwide repository for biomolecular simulations

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BioSimGrid is a distributed database for biomolecular simulations, or, a 'Protein Data Bank extended in time' for molecular dynamics trajectories. We describe the implementation details: architecture, data schema, deposition, and analysis modules. We outline some examples, among many more, where BioSimGrid is a necessary tool: The first is the comparison of several esterase simulations, including those of acetylcholinesterase (a key enzyme in the nervous system) and bacterial outer-membrane phospholipase (a bacterial enzyme involved in pathogenesis). Structural data show that these enzymes have similar active sites; the structures of the proteins are otherwise unrelated. The second involves a set of high-throughput outer-membrane protein simulations, with analyses focusing on the interactions between the protein and lipid molecules. We encourage the simulation community to explore BioSimGrid and work towards a common trajectory exchange format.

Posters
– Modelling Molecules –

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Theoretical IR spectroscopy by QM/MM calculations support the modelling of the RAS-GAP-GTP complex

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The GTPase Ras p21 is a crucial switch in cellular signal transduction. The GTPase-activating protein (GAP) has the important role to deactivate the complex by accelerated GTP hydrolysis.
FTIR spectra of the substrate guanosine triphosphate (GTP) show remarkable changes when it binds to the enzyme. Computationally we can observe these spectral features in our QM/MM simulations. We have simulated Ras·GTP in solution and isolated GTP in water. The triphosphate part of GTP was treated quantum mechanically using DFT. Vibrational spectra were calculated in harmonic approximation with an average deviation of 3% from the experimental frequencies.
The established method of theoretical IR spectroscopy is used to support the modelling of the so far unknown enzyme-substrate complex Ras·GAP·GTP. To this end, the known Ras·GAP·GDP·AlF3 structure (1WQ1) is chosen as a starting point. The coordination of GAP's Arg789 to the triphosphate is studied in detail as the arginine-finger is the main part of GAP influencing the GTP hydrolysis. The calculated phosphate bands turn out to be very sensitive to the coordination of Arg789 to the triphosphate group. Comparison with experimental IR spectra allows rational design of structural details.

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Negatively cooperative binding of melittin to neutral phospholipid vesicles

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The association of basic amphipathic peptides to neutral phospholipid membranes is studied with binding and partition models. The binding of native and modified melittin (Mel) to egg-yolk phosphatidylcholine (EPC) is studied by spectrofluorimetry and size-exclussion chromatography (SEC). Results: (1) The binding isotherms for DNCMel and Mel to EPC. (2) The Scatchard plot for the binding of DNCMel and Mel to EPC. (3) Hill plots at fixed peptide concentrations. (4) The dissociation constant $pK_D{}^h$ calculated from pK_D at each site with Hill coefficient h. (5) The partition coefficient Γ for the binding of DNC–SP to EPC and of mastoparan to dioleoylphosphatidylcholine (DOPC). (6) $K_D{}^{h}$–^{ho} *vs*. ionic strength I plot. Provisional conclusions: (1) SEC is proved useful for quantifying the polyelectrolyte–lipid association. (2) Spectrofluorimetry suggests a similar lipid-bound state for native and modified Mel. (3) Gouy–Chapman and Debye–Hückel formalisms apply the Poisson–Boltzmann equation for the influence either of a plane charged surface or between two charged spheres over the structure of the adjacent ionized liquid. (4) $pK_D{}^h$ in the binding of native and modified Mel to EPC is dependent on h. (5) Coulombic repulsions drop more than peptide–lipid attractive forces with I, enhancing association.

P-651

MD simulations of nanotube-membrane interactions and an olygopeptide transmembrane penetration

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Molecular dynamics simulation of a complex system comprised of a hydrated lipid membrane, nanotube "soldered" at one end and certain molecule to be pushed through the membrane. A constant force in normal direction to membrane is applied to pentadecamerous polyalanine located in the channel of carbon nanotube adjoining a lipid bilayer at right angle. Under the action of the force the oligopeptide gets into the membrane. This construction can be considered as a delivery vehicle which drives the peptide to the membrane surface. As a matter of principle, tuning the nanotube (by adding of functional groups) one may achieve the selectivity of nanotube's landing area on the cellular membrane. The pressure expulsing the peptide could arise as a result of the chemical reaction which makes the reaction mixture volume increase in the soldered nanotube. The chemical agents start reaction under the action of certain signal (for instance, a flash of light), and during the time in the order of a nanosecond the peptide finds oneself inside a cell. Of course, only some features can be reflected in MD calculations so far. Nonetheless, the reported simulation is a first step on the road to construction of such complicated biomimetic systems. Some regimes of penetrations are considered with regard to different molecular timescales.
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P-652

Elastic lever-arm model for myosin V

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We present a model for processive motion of myosin V. We describe the dimeric molecule as two identical heads, connected with elastic lever arms. We show in a quantitative fashion why the lead head only binds to actin after the power stroke in the trail head and why it undergoes its power stroke after the release of the trail head. This provides an explanation for the coordinated hand-over-hand motion [1]. We calculate the distribution of step sizes for different lever arm lengths. The best agreement with observed (EM) distributions is achieved when fluctuations in the actin helix are taken into account. We also show how processivity studies could help determining the kinetic rates of an individual head.
[1] A. Vilfan, Elastic lever-arm model for myosin V, Biophys. J. **88**, *in press* (2005), doi:10.1529/biophysj.104.046763

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– Modelling Molecules –

P-653

Structure prediction of Dardarin, the PARK-8 Parkinson´s disease susceptibility protein

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Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting up to 50% of individuals over age 85. At present, PD is incurable and its etiology is unclear. Much hope for obtaining a better understanding of the molecular basis of this disorder is vested in efforts to identify genes linked to familiar forms of PD. The latest gene is the leucine rich repeat kinase 2 (LRRK2) also known as dardarin.
Despite the very strong evidence that dardarin is a key player in causing Parkinson's disease nothing is known about its biological function.
Using comparative modelling techniques models were made for the 2527 amino acid sequence of dardarin. Using sliding windows we succeeded in delineating the different folding domains in this protein. A detailed structure was made for the important domains and the influence of the mutations was simulated. Furthermore a hypothesis was formulated how the different domains cooperate to fulfill the function of dardarin. Finally the influence of the pathological mutations on the activity has been studied.

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Study on the Interaction between pi-electron of Benzene and Waters Using QM/MM Simulation

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Weak non-covalent interactions are important in constructing protein structures. In many protein structures, a non-covalent weak force has been noticed to act between the π-electron cloud as a hydrogen bond acceptor and hydrogen as the donor, and the compact protein core conformations are formed. An approach by the classical molecular mechanics (MM) cannot attain to the quantitative analysis of such weak interactions associated with the π-electrons. We applied the QM(quantum mechanics)/ MM molecular dynamics simulation to a system composed of a benzene molecule in water. The benzene as an acceptor of hydrogen bond and a water molecule as a donor of that were treated by QM, and the other water molecules were done by MM. Simulation, in which the system of benzene-waters as QM was solvated by explicit TIP3P water models with an isotropic and periodic boundary condition, was carried out in a canonical ensemble. The QM calculations were performed with a hybrid ab initio RHF and DFT. Results indicated that the hydrogen atom of the water molecule in the hydration layer locates closely just above the benzene ring with meaningfully high probability, forming a hydrogen bond with the π-electron. On the contrary, in conventional molecular dynamics simulations, any water molecules did not form distinct hydrogen bonds with the benzene ring.

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Iron electronic structure of the heme models for alfa– and beta–subunits in deoxyhemoglobin

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Cooperative oxygen binding by tetrameric hemoglobins is determined by the presence of two pairs of non–identical protein subunits. Human adult hemoglobin consists of two α– and two β–subunits. The X–ray structure analysis of human adult hemoglobin revealed stereochemical differences of the heme iron in α– and β–subunits in tetramer. It is possible that the heme iron electronic structure non–equivalence in α– and β–subunits may also play a special role in cooperative oxygen binding. Preliminary *ab initio* calculations of the heme iron electronic structure were made using Xα discrete variation method for deoxy–heme models. These models were based on the X–ray structure analysis of human adult deoxyhemoglobin (Fermi at al., 1984) and consist of 53 atoms with coordinates obtained from the PDB. The differences of the iron electronic structure were calculated for these models. Moreover, calculated temperature dependences of quadrupole splitting for the models of α– and β–subunits were also different.

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Survival/proliferation or apoptosis/necrosis, seen in oxidative stress exposed human dendritic cells

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Signaling mechanisms that utilize reactive oxygen species to initiate processes that allow cells to survive exposure to oxidative stress, or to actively die, when stress induced damage is too great, are in many respects insufficiently elucidated. In particular role of oxidative stress in dendritic cell activation/inhibition is far from being delineated. The aim of this study was to test hydrogen peroxide effects on human peripheral blood monocyte derived dendritic cell populations in various culture conditions.
Selecting appropriate molecular probes (PI, Hoechst, AnnexinV-FITC), changes in cell viability, proliferation rate, percentage of apoptotic and necrotic cells were assessed by fluorescence microscopy, and flow cytometry. Phase contrast microscopy was used for morphologic inspection, and intracellular calcium level changes were documented using Fluo-3 as molecular reporter. Modulation of the observed changes by exogeneous arachidonic acid in micro- and milimolar concentrations, also was followed up.
The relation of these findings to possible in vivo mechanisms of dendritic cell regulation is considered.
Partial financial support of the Romanian Ministry of Education and Research (VIASAN 178/2002) is gratefully acknowledged.

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The effects of occupational exposure to X-rays in blood of radiographers

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The aim of this study is to investigate the risk of occupational exposure to X-rays in blood of radiographers. The data obtained on hematological levels revealed insignificant changes in erythocytic counts, platelate and hemoglobin concentration due to occupational exposure of X-rays. Significant decrease was recorded in packed cell volume (PVC) due to exposure of x-rays. The data revealed that exposure to X-rays caused significant increase in serum creatinine, uric acid and bilirubin concentrations.

P-658

Irradiation activity of some plants grown in volcanic regions

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The paper presents a study of radioactivity of some plants grown in volcanic regions of Romania, namely in neighbourhood of Easter Charpatian Harghita mountain, in comparison of a distant zone, over 100 km, Central Transylvania. The radioactivity of plants arise from radionuclids absorbed from soil, water and air.
The geology of Harghita mountain is mainly composed of andesitic agglomerate which are mixtures of andesitic lava and various sized pyroclastic rock fragments such as quartzites, schists, gneisses, basalts, andesites and rhyolites. Tha rock can be ranged into albite – orthoclase – anortit system, zone Labrador – andesine, as a complex alkali – alkali earth silicate basaltic – andesite.
The radionuclids are present in soil in the countryside originate from degradation of volcanic rocks. The compact volcanic rock presents an activity 5-times higher then the soil of region (1 count per second / 0.2 count per second). The distribution of radionuclids evaluated with increasing thickness of soil up to 0.3 m, presents a difference regarding of soil composition. The topsoil presents a crescent intent of activity up to 10 cm, then present decreasing values. The clay content soil has a permanent descending value of activity with increasing thickness. The plants grown in this area presents a differing radioactivity in comparison of control plants grown in 100 km distance from volcanic region.

P-659

Meso-tetrakis (4-sulfonatophenyl) porphyrin-phenothiazine interactions: spectroscopic studies

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Water-soluble meso-tetrakis (4-sulfonatophenyl porphyrin ($TPPS_4$) has been studied extensively related to its potential use in biomedical applications. It has the ability to form the diprotonated species at acidic pH leading to porphyrin aggregates with characteristic spectroscopic features. In the present work the interaction of $TPPS_4$ with two phenothiazine derivatives, trifluoperazine (TFP) and chloropromazine (CPZ) was studied through the use of optical absorption, emission and resonant light scattering spectroscopies. Titration of solutions of $TPPS_4$, at pH 3.0 and pH 7.5, has shown dramatic changes of optical absorption spectra suggesting formation of a porphyrin-phenothiazine complex. Fluorescence emission of $TPPS_4$ is severely quenched by addition of phenothiazines and the Stern-Volmer plots are non-linear. Finally, resonant light scattering intensity is significantly increased suggesting that a complex involving a considerable number of molecules is produced. All the effects described above are more intense at pH 7.5, where $TPPS_4$ bears a total of 4 negative charges and are quite reduced at pH 3.0 due to the reduction of its anionic character upon protonation. The effects of TFP seem to occur at relatively lower concentrations as compared to CPZ supporting its higher hydrophobicity. Support: PET-SESu/MEC, CNPq.

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Translocation of cephalexin antibiotic through OmpF porin channel

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The resistance to a variety of antibiotics is the major problems in the control of pathogenic bacteria that can be partially overcome by facilitating the antibiotics passage through the membrane channels. Tuberculosis is of the most challenging diseases that is caused due to multidrug resistant (MDR) properties of the *Mycobacterium tuberculosis*. Biophysical investigation of the passage of single antibiotic through single channel in real time can reveal the physicochemical conditions required to manipulate and facilitate the passage of the drug at molecular level.
In this project we have studied the passage of cephalexin antibiotic through OmpF channels reconstituted in lecithin planar bilayer. Most of the experiment were conducted when only one single channel was present in the bilayer where its gating, conductance and voltage sensitivity was analyzed in the presence and absence of antibiotic.
The study revealed that cephalexin decreased the channel conductance by about 30%. Furthermore, the voltage sensitivity of the channel was decreased by about 40%. Channel gating and the interaction between cephalexin and channel pore at molecular level will be discussed in this article.

P-661

Micronucleus Incidence In Individuals Professionally Exposed To Ionizing Radiations

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Effects of chronic exposure to low level ionizing radiations (as is the case of professional exposure) are poorly understood and result in latent injuries of the chromosomal genetic material propagating to next cell generations.
Our study is an individualized radiation-induced genomic instability risk assessment in a population professionally exposed to low dose ionizing radiation as evidenced by micronucleus (MN) incidence in cultured lymphocytes in arrested cytokinesis.
In a sample of about 500 healthy adult workers of both sexes aged 23-52, less than 10 had more than 20 MN/1000 binucleated cells, most of which being heavy smoking women. No correlation with age or sex could be found. The MN incidence found is close to the figures reported by the HUMN project and the proportion of less than 2% cases with more than 20 MN/1000 binucleated cells is a reasonably good figure for the considered group. Lack of MN incidence data in unexposed Romanian population blocks comparisons of our findings with baseline figures.
A definite requirement for the evaluation of the baseline in the general population arises and in order to allow interpolation of our findings in a larger European context we intend to participate in the HUMN project.

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Purification, crystallization, and preliminary crystallographic analysis of a new lipase

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Microbial lipases that catalyze the hydrolysis of long-chain acylglycerols into glycerol and fatty acids attract considerable interest partly because of their industrial applications such as in detergents, oil/fat/cheese production, leather/paper industries, and industrial synthesis of fine chemicals. A lipase secreted by Penicillium expansum PF898 (PEL) was cloned and was found to have low (up to 28%) sequence identity to any known lipases. This lipase was overexpressed in Pichia pastoris with high yield and purified to homogeneity. This purified lipase was then crystallized at room temperature by sitting-drop vapor diffusion method using ammonia sulfate as main precipitant. The lipase crystals diffracted to 1.7 Å with an in-house copper rotating-anode X-ray generator. A 2.08Å data set has been collected, indicating the crystal belongs to space group either P43212 or P41212, with unit-cell parameters a = 88.09Å, b = 88.09Å, c = 126.54Å. The asymmetric unit contains 1 molecule and has a solvent content of ∼44%. The structural determination is currently underway using molecular replacement, multiple isomorphous replacement, and single-wavelength anomalous scattering methods.

P-663

Antioxidative and membrane activity of some novel N-Oxides

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There is a continuous search for new compounds that can be applied biologically in different character. Some of them are used as antioxidants and for that purpose a series of *N*-oxides of tertiary amines (NTA) presented was synthesized. Individual compounds differed in the alkyl chain length (C_nH_{2n+1}; n = 11, 13 and 15).
Various methods were used to check their antioxidative efficiencies. These were studies on erythrocyte membrane lipid oxidation induced by UV irradiation in the absence (control) and presence of NTA and chromogen experiments enabling to compare compounds' antioxidative efficiencies with that of Trolox (vitamin E analogue). In parallel, some experiments were performed to determine the mechanism of the interaction between NTA and membranes and degree of membrane structure disordering. The measure of these were hemolysis (erythrocytes) and chlorophyll content, potassium leakage and plant growth inhibition (cucumber hypocotyls).
It was found that NTA studied exhibited sufficient activity to be used as antioxidants. The best antioxidative activity was found for $C_{11}H_{22}$ alkyl chain compound. This compound was also found most active in the plant experiments.
The work was sponsored by the Polish Research Committee (KBN), grant no. 2 P04G 088 27.

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Influence of the integrity of the corneo-epidermal interface on the biophysical properties of the skin

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The hydration and biophysical properties of the skin are functions of a well-differentiated epidermis and a water impermeable horny layer. Tight junction proteins (claudin-1) are key proteins in the control of the watertightness of the interface between the horny layer and the epidermis (the corneo-epidermal junction). Aquaporin-3 (AQP-3) forms molecular transmembrane water channels involved in epidermal water diffusion. We obtained biopsies of facial skin from Japanese women (20-78y old) undergoing plastic surgery. Samples were fixed and embedded in paraffin. Histological studies showed that the epidermis becomes thinner with age (y = 64.4-0.3x), mainly affecting the areas most exposed to sunlight. This was linked to a decrease in the keratinocyte layers (p=0.01). Anti-claudin-1 antibody mainly stained the upper granular layers and the amount of claudin-1 was positively correlated to AQP-3 in the human epidermis (plasma membrane staining). In addition, the amounts of claudin-1 and AQP-3 decreased with age (y = 9.4-0.04x; y = 20.6-0.1x respectively). A natural mixture of uronic acid + rhamnogalacturonanes on human normal keratinocytes grown in SFM supplemented with EGF was tested. Claudin-1 mRNA was extracted and amplified by quantitative RT-PCR. Claudin-1 gene expression was increased significantly (up to 50 fold). The regulation of claudin-1 at the corneo-epidermal junction by natural molecules is a new way of controlling epidermal skin hydration.

P-665

G-quadruplexes as molecular beacons

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Molecular beacons are stem-loop probes designed to hybridize to a specific complementary target. A G-quartet is a planar association of four guanines held together by 8 hydrogen bonds and G-quadruplexes result from the hydrophobic stacking of several quartets. G-quartets, which have been discovered decades ago, now have applications in areas ranging from supramolecular chemistry to medicinal chemistry. We are currently investigating the use of G-quadruplexes for the stem of molecular beacons, replacing the classical watson-crick complementary stem. We first demonstrate that an intramolecular G-quadruplex may accomodate the long (>10 bases) central loop, necessary for sequence complementarity with the target. The stability of such a quadruplex is only weakly affected as compared to a classical quadruplex, such as $(GGGTTA)_3GGG$. These new G4-based molecular beacons exhibit an exquisite sequence specificity: a single base change in the sequence of their target led to a strong destabilization of the complex, allowing an excellent sequence discrimination. The thermodynamic and kinetic parameters characterizing this match /mismatch discrimination have been determined using a combination of thermal melting and stopflowed kinetics experiments.

P-666

Physicochemical characterization and endotoxic activity of a rhamnolipid from Burkholderia

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A highly purified glycolipid, a diacylated rhamnolipid produced by *Burkholderia plantarii*, was analyzed physicochemically and in biological test systems with respect to its pathophysiological activities as heat-stable extracellular toxin. FTIR was applied to study specific molecular groups, synchrotron radiation X-ray diffraction to elucidate the aggregate structure, fluorescence resonance energy spectroscopy to test the ability of the RL-2,2_{14} to intercalate into phospholipid liposomes in the absence and presence of lipopolysaccharide-binding protein, and the biological activity of RL-2,2_{14} was examined as the ability to induce cytokines in human mononuclear cells.Despite its completely different chemical structure, the rhamnolipid RL-2,2_{14} exhibits a variety of endotoxin-related physicochemical characteristics such as cubic inverted aggregates and the ability to induce cytokines in MNC. These data are in good agreement with our conformational concept of endotoxicity, based on the intercalation of naturally-originating virulence factors, these are beside rhamnolipids endotoxin (lipopolysaccharide), glycolipid from *Mycoplasma fermentans* (MfGL-II) and also synthetic lipid A-mimicking structures expressing the "endotoxic conformation" intomembranes of immune cells, leading to strong mechanical stress on integral proteins eventually causing cell activation.

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Polymyxin B and its nonapeptide binding characteristics to endotoxins as investigated by calorimetry

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The interaction between endotoxins – free lipid A and various lipopolysaccharide (LPS) chemotypes with different sugar chain lengths – and the polycationic peptides polymyxin B (PMB) and polymyxin nonapeptide (PMBN) has been investigated by isothermal titration calorimetry (ITC) between 20 and 50 °C. The results show a strong dependence of the titration curves on the phase state of the endotoxins. In the gel phase (< 30 °C for LPS and < 45 °C for lipid A) an endothermic reaction is observed, for which the driving force is an entropically driven endotoxin-polymyxin interaction, due to disruption of the ordered water structure and cation assembly in the lipid A backbone and adjacent molecules. In the liquid crystalline phase (> 35 °C for LPS and >47 °C for lipid A) an exothermic reaction takes place, which is mainly due to the strong electrostatic interaction of the polymyxins with the negative charges of the endotoxins, i.e., the entropic change ΔS is much lower than in the gel phase. For endotoxins with short sugar chains (lipid A, LPS Re, LPS Rc) the stoichiometry of the polymyxin binding corresponds to pure charge neutralization, for the compounds with longer sugar chains (LPS Ra, LPS S-form) this is no longer valid. This can be related to the lower susceptibility of the corresponding bacterial strains to antibiotics.

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Effect of baicalin on erythrocyte plasma membrane of hypercholesteolenic patients

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The radix of *Scutellaria baicalensis Georgi* is a traditional herb medicine in China and Japan. One of the major flavonoids is baicalin, which has antioxidant, anti-inflammatory and anticancer activities. The purpose of the study was to evaluate the effect of baikalin *in vitro* on lipid peroxidation and the cholesterol in the erythrocyte membranes of patients with non-treated hypercholesterolemia. The patients were selected on the basis of the plasma total cholesterol higher than 250 mg/dl, LDL-cholesterol> 170 mg/dl and triglicerydes level not exceeding 400 mg/dl. Whole blood and erythrocytes with hematocrit 2% were incubated with 100 ìM baikalin for 24 hours at 37°C. The higher concentrations of cholesterol and TBARS in erythrocyte plasma membrane of patients with hypercholesterolemia were observed. After incubation with baikalin the amount of cholesterol and TBARS decreased. No significant changes in control group were observed. Lower ATP-ase activity in erythrocyte plasma membrane of patients with hypercholesterolemia was found. Further decrease in the activity of the enzyme was observed, after incubation of erythrocytes with baicalin.

P-669

Characterization of membrane activity of and lesion formation by the antimicrobial hBD3

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We investigated the role of the composition of the lipid matrix, e.g. the presence of negatively charged lipids, of sensitive and resistant Gram-negative bacteria for the activity of the polycationic human β-defensin-3 (hBD3), an antimicrobial peptide of the innate host defense. A correlation could be found between the biological activity of hBD3 determined in biological assays and data obtained from biophysical experiments utilizing lipid bilayers as membrane reconstitution systems. Membranes were reconstituted using lipids isolated from the respective bacterial strains. hBD3 intercalated only into those bilayers resembling membranes of sensitive bacteria and destabilized them by the formation of lesions. We propose that the lipid specificity of hBD3 and other membrane-active antimicrobial peptides is the decisive first step in its antimicrobial activity. To obtain more details on the lesion formation process, we determined the current/voltage characteristics of the lesions as well as time-based standard deviations and Fourier transformations at particular time points of the current traces. These data were compared with those of polymyxin B-induced pores and bacterial porins. From this analysis it is evident that hBD3 induces lesions rather than defined pores.

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Effects of antisense anti-MDM2 oligonucleotides on proliferation and apoptosis of BEAS-2B cells transformed by γ ray irradiation

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Objective: The MDM2 oncogene has been suggested to be a novel target for cancer therapy. It is reported that antihuman-MDM2 ASON has anti-tumor activity when administered alone or in combination with other cancer chemotherapeutic agents. This study was undertaken to investigate the effects of MDM2 ASON on proliferation and apoptosis of BEAS-2B cells transformed by γ ray irradiation.
Methods: Expression of MDM2 after treatment with ASON and its mismatch control were tested with western blot and RT-PCR assay. The effects of MDM2 ASON to proliferation and apoptosis of BEAS-2B transformed cell were determined with MTT, ^{3}H-TdR incorporation and FACS analysis assay.
Results: After specific inhibition of MDM2 expression, the ASON, in a sequence-specific manner, significantly inhibited the proliferation of BEAS-2B cell and increased TNF-induced apoptosis rate. Conclusion: These results suggest that MDM2 has a role in mediation on proliferation and apoptosis of BEAS-2B cell, providing a basis for future development of anti-MDM2 ASON as cancer therapeutic agents used alone or in combination with conventional chemotherapeutics.

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Kir2.1 potassium channel measurements with a 4Pi-microscope: Imaging, Deconvolution, Quantification

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Strong inwardly rectifying potassium channels of the Kir2.x family play a central role in the regulation of cellular excitability in the brain and in the heart. Mutations in Kir2.1 cause Andersen's Syndrome, a hereditary disease characterised by periodic paralysis, cardiac arrhythmia and dysmorphic features. The 4Pi microscopy technique allows a significant improvement of the axial resolution of light optical measurements by the use of interference of wavefronts focused by two opposing lenses compared to confocal microscopy. Due to technical difficulties that have been resolved recently, 4Pi microscopy has to date rarely been applied for the study of mammalian cells. We have for the first time successfully used 4Pi microscopy to measure characteristics of Kir2.1 ion channel clustering in the cell membrane of mammalian HEK293 cells at a spatial resolution of 100 nm in all three axes. We show that Kir2.1 channels are concentrated in large clusters that are distributed homogenously over the cell membrane. The clusters have a rather cylindric shape with the long axis along the optical axis of the measurements. The size of a single cluster could be measured. In summary, we have shown that 4Pi microscopy can be applied to study the morphology of cell membrane structures at nanoscopic resolution. The measurements are done with the commercial 4Pi-microscope constructed by Leica-microsystems, Mannheim, Germany.

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Ion channels and disease: cellular and biophysical properties of P/Q-type calcium channels in SCA6

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The molecular basis of neuronal dysfunction in spinocerebellar ataxia type 6 (SCA6) is poorly understood. SCA6 is a trinucleotide repeat disorder that affects the neuronal voltage dependent P/Q-type calcium channel (CaV2.1). The disorder may arise due to altered calcium channel function (channelopathy), due to the cellular consequences of the polyglutamine expansion at the channel C-terminus (glutaminopathy) or by a combination of both mechanisms. Here we transfected cells with either with CAG_{11} (WT); CAG_{23}, CAG_{72} (SCA6); or EGFP and compared to untrasfected controls. Channels were expressed in non-excitable HEK293 cells and/or in cultured cerebellar granule neurons (CGN) from P/Q-type channel KO mice.
We will present data on the biophysical properties of WT and SCA6 P/Q-type channels. In particular, the ability of SCA6 channels to interact with β_4 subunits was explored in HEK293 cells. β_4 subunits interact with the α subunit C-terminal region near the mutation locus in SCA6. Calcium current kinetics, calcium- and voltage-dependent inactivation; and calcium-dependent facilitation were quantified in SCA6 and WT channels.
Second, to determine whether calcium channel activity affects neuronal survival in SCA6, we used genomic DNA degradation to quantify the calcium channel influence in the degree of programmed cell-death in CGN.
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Effect of electromagnetic field irradiating from high-voltage transmission line on serum´s biochemical and blood index of animals

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Effects of low-frequency electromagnetic field(EMF) of highvoltage transmission lines(HVTL) on Serum Biochemical and Blood general Index of rates are measured. The rats in 4th weeks are divided into controlled and exposed groups. The latter is exposed in EMF about 400days. The EMF is generated by an appliance. Their strengths of the electric and magnetic fields are 4000V/m and 0.09-0.1G, respectively, which are that at the position of 2.0- 2.3 m distancing the earth under the HVTL of 220KV. We measure some biochemical indexes of serum by LQ-300K instrument and the numbers of erythrocyte, leucocyte and hemalbumin. Through comparison we find that ALT increases ($P<0.05$) and leucocyte increases obviously in the experiment group ($P<0.01$). We measure electromagnetic features of the serum, and find that refraction index and dielectric constant increase from 1.3353 and 1.7830 for the controlled group to 1.3361 and 1.7852 for exposed group, respectively. We measure also their spectra of infrared absorption by FT-IR 670 spectrometer and found that the spectra of the controlled and exposed groups are different both intensity and frequency. This shows that structure of the molecules in the bloods changes under influence of EMF of HVTL, which results just in above changes of the serum's biochemical and blood general index and electromagnetic features of animals.

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A putative polyketide synthesis protein XC5357 from a plant pathogen Xanthomonas campestris

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Microorganisms and plants synthesize a large variety of polyketide metabolites, many of which are medically important antibiotics or exhibit other pharmacological, such as antibacterial, antivirals, or antitumor activities.
Tetracenomycin (TCM) C is a cytotoxic antibiotic produced by *Streptomyces glaucescens* and is notable for its broad activity against actinomycetes. Its synthesis is currently one of the models for the antibiotic production in *Streptomyces* species. Sequence analysis of the entire TCM C gene cluster *tcmGHIJKLMNO* operon has been completed, and a model for the Tcm PKS catalyzed synthesis of the TCM C has also been set up. Reconstitution experiment of the PKS for TCM F2, a precursor of TCM C, suggests that the active PKS complex consists of at least four major proteins, including the Tcm*K*. *–L*, *-M*, and *–N* gene products. Tcm*J* is also a one of the components of the PKS complex, but its function is currently unknown, although its addition to the Tcm*KLMN* complex can greatly increase the production of TCM F2 by nearly fourfold.
XC5357 from the plant pathogen *Xanthomonas campestris* pv. campestris is classified as a polyketide synthesis protein from a bioinformatics approach. It contains 113 amino acids, and shares an 82 % identity with a similar protein in the *Xanthomonas axonopodis* pv. *citri*, and a 30% identity with the Tcm*J* proteins from the *Streptomyces glaucescens* and *Streptomyces coelicolor*. In this report, we describe the cloning, purification, crystallization, and X-ray analysis of XC5357.

P-675

Ferrofluids-nanoparticulate systems to deliver drugs in solid tumours

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Magnetic drug targeting is a method for targeted delivery of drugs in a tumour area using external high -gradient magnetic fields.This has the potential advantage of an increase in local drug efficiency and in a reduced toxicity concerning the entire body.The drug delivery system is in this case a biocompatible ferrofluid -colloid of magnetic nanoparticles dispersed in a carrier liquid. Experiments on animals have already proved the efficiency of the method when no viable tumour tissue was histologically evident.[1,2]Most critical are the biocompatibility , magnetic susceptibility , size of the particles and in vivo drug desorption from nanoparticles.Large particles or aggregates due to chemical or magnetic reasons can lead to embolic problems.The ferrofluids we investigate are prepared in our laboratory by coprecipitation from iron- citrate mixtures. Stability and biocompatibility are assured through a citrate shell exchanged with phosphodextrane for the basic drug load mitoxantrone.The determination of the conditions for safe size distribution which would avoid embolism has priority. In order to balance the capture in magnetic field against blood flow, a high enough magnetic moment is required.This will introduce a compromise in having very small nanoparticles. For these purposes the ferrofluids are investigated using different techniques: TEM, DLS , Powder diffraction , Mößbauer, EPR and Vibrating Sample Magnetometry.
[1] C.Alexiou et al JMMM 252 (2002) 363-366
[2] C Alexiou et al Cancer Research 60 (2000) 6641-6648

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P-676

A critical assessment of the information provided by ^{1}H-NMR spectroscopy in diabetic urine analysis

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Introduction: Diabetes mellitus, along its associated complications, is one of the important cause of death worldwide. H-NMR has already proven its power in the urine analysis.
Aim: The present study compares the urine profile in both normal and type II diabetic subjects, in order to identify the degree of the progression of renal diabetic insult.
Materials and Methods: NMR spectra have been recorded on a Bruker Avance DRX 400 MHz spectrometer. The control group (no=26, 36 $\pm$11 years old), has no evidence of a renal disease. The type II diabetic group (no=15, 48$\pm$12 years old) was recruited from patients with normal metabolic control of the disease. Data are given in mmol/mol creatinine, $p<0.05$ was taken as significant.
Results: the average of various metabolites in urine for diabetic patients is significantly different from that of the control group (e.g. alanine 85.7 vs 44.9, GABA 149.3 vs. 104.3, piruvate 63.06 vs 41.6, TMAO 147.7 vs. 37.7, etc.). The interval over which the individual values are spread overlaps for all metabolites.
Conclusions: NMR provides valuable information on the biochemistry and mechanism associated with diabetes. The interpretation of any metabolite values as an early marker of the disease is possible for the cases with extreme values, that don't interfere with the control group.

P-677

Aggregational state of polymyxin analogues in the membrane: a fluorescence based approach

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Despite the emergence of bacterial resistance to the majority of antibiotic classes, acquired resistance to polymyxin has not been described. This is most likely related to its mode of action as a bacterial membrane perturbant, a molecular mechanism for which it is difficult for organisms to mount a counter response in terms of mutational resistance. Although polymyxin B (PxB) causes adverse effects on man, the discovery of new agents with a related mode of action may generate antibiotics with minimal potential for drug resistance. Our approach is to dessign and synthetize novel cyclic peptides related to PxB that have improved properties as potential drugs. In this study, the interaction of two synthetic PxB-analogues (sP-Trp; sP-pyr) with lipid membranes was investigated. sP-Trp incorporates a *(D)*Trp residue instead of *(D)*Phe6 of PxB, and sP-pyr is labelled with a pirene group at the N-terminus. Using intermolecular FRET bewteen Trp as donnor and pyrene as acceptor, the aggregational state of the peptide in the membrane was determined. Also, intrinsic Trp fluorescence emission and quenching by acrylamide was used to characterize the interaction of the peptide with the membrane.

P-678

Complex analysis of human tear secretion – diagnosis method of ocular diseases

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Objectives: This study analyses electrophoretic patterns of tear proteins from normal subjects and patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) and identifies the level of lysozyme and lactoferrin.
Methods: Subjects repartition was: a SLE group (n=8), a rheumatoid arthritis group (n=9) and a control group of healthy volunteers (n=10). Tears were collected using the Schirmer's method. Proteins were separated by sodium-dodecyl sulfate poliacrylamide gels electrophoresis. The lanes were evidenced by Coomassie blue and/or silver staining. Western blot analysis assay was used to identify lysozyme.
Results: Lactoferrin, lysozyme, tear specific pre-albumin and transferrin were found to be main components being identified using molecular weight markers. The tear proteins patterns of some patients with rheumatic diseases are different from those of healthy subjects. There were subjects with a decreased level of lysozyme and lactoferrin that can indicate a secondary Sjogren's syndrome(SS) clinically undiagnosed.
Conclusions: Electrophoretic analysis of tear protein patterns is a fast, reproducible and simple method that could provide information for a precocious diagnosis of SS.

P-679

Glutamine- and Asparagine-rich proteins and misfolding diseases

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The function of most proteins depends on a well-defined average three dimensional structure and protein misfolding is associated with many different neurodegenerative diseases. It has been established that proteins rich in the amino acids Glutamine (Gln) and Asparagine (Asn) have a greater propensity to spontaneously form the amyloid aggregates characteristic of misfolding diseases [1]. Here this aspect of their chemical composition is used to explain why these proteins are more prone to misfold than normal proteins. The general idea is that vibrational excited states are the drivers of protein folding and function and Gln and Asn are the only two amino acids that can interfere with the process of vibrational energy transfer in proteins. Computer simulations with an extended version of The Davydov/Scott model [2,3] demonstrate the possibility of energy transfer from the water solution to the protein and show that Gln and Asn residues lead to an initial larger absorption of energy from the environment to the protein, something that can explain the greater structural instability of Gln-rich and Asn-rich proteins. The sporadic, inherited and infectious character of prion diseases is discussed and an alternative treatment for misfolding diseases is suggested.
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P-680

The mode of action of bicomponent gamma-hemolysins of Staphylococcus aureus

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The bicomponent gamma-hemolysins of *S. aureus* (HlgA, HlgB, HlgC) are included in the beta-barrel pore-forming family, that also includes Panton-Valentine leucocidin and alpha-hemolysin. Toxins belonging to this group oligomerize on cell and model membranes and open a pore in the lipid. Alpha-HL pores are formed by seven identical protomers, whereas bicomponent toxins are assembled associating two different proteins (e.g. AB or CB) taken from two different sub-families (class S and class F)
Based on FRET between couples of labelled mutants, evidences for an alternate topology (of the type ABABAB) of the active pore will be presented. This result is further supported by inhibitory experiments performed with preassembled AB dimers. The single component (either A or B) is able to reduce the ability of dimers to form pores both on artificial membranes and on rabbit erythrocytes.
The 1:1 stoichiometry of the pore has been evidenced by experiments of conductance on planar lipid membranes, which have been performed at different AB or CB ratios.
The interaction of the gamma-hemolysins with lipid has been finally investigated. We found that the pore-forming ability is directly related to the cholesterol availability.
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P-681

A magnetic man

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Approximately one year ago, we were surprised because of a TV program. A man has been claiming and presenting himself as a magnetic man. His naked chest really had been pulling metal objects as a magnet. The metal objects had been really sticking skin of his chest. Therefore, the purpose of this study is to examine this man.
After he gently accepted to be voluntar subject of this study, we firstly measured magnetic field of his body by a small magnetic field meter, which is called as cell sensor. The measurements showed that there is a magnetic field at mG level around his chest. In the second step, we measured EEG, EKG, EMG, evoked potentials, bone mineral densitometry, total biochemistry parameters, blood parameters, and hormones such as calcitonin, ANTI TPO, ACTH, Cortisol, and PTH. On the other hand, we measured trace elements and T1- T2 relaxation time in blood serum.
Adittionally, 17 parameter such as resistance (R), reactance (X), phase angle (α), body capacitance (C), fat-free mass (FFM), body cell mass (BCM), extra cellular mass (ECM), fat mass (FM), ECM/BCM, body mass index (BMI), basal metabolic rate (BMR), total body water (TBW), intracellular water (ICW), extra cellular water (ECW), TBW/Fat-free mass, TBW/Total weight were measured by means of Bioelectrical Impedance Analysis (BIA).
Magnetic field measurements and metal interaction with his chest were recorded by a video camera. Finally, we will present recorded images and results in BioEm2005.

P-682

Characterisation of the Cu(II) binding sites in the N-terminal domain of the prion protein

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Transmissible spongiform encephalopathies (TSEs) in mammals are neurodegenerative diseases caused by an infectious agent called prion, which seems to be exclusively composed of the disease-related isoform PrP^{Sc} of the cellular prion protein PrP^{C}. Over the past few years, research work has demonstrated that the prion protein is a copper binding protein. Several studies have focused in the interaction of the prion protein with copper in the N-terminal domain of the protein, specifically in the octarepeat region, which consists of four tandem repeats of the sequence PHGGGWGQ.
The present study is addressed to infer the solution-state geometry of the tetraoctapeptid-Cu^{2+} complex and its pH dependence. To investigate the geometry of the peptide-Cu^{2+} complex, a new method for structure determination of the copper environment is used. This method combines molecular dynamics computations with spectroscopic data from electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR). Computations are used to select sterically possible structures which are sorted attending to certain restraints inferred from the spectroscopic data. The selected structures are analyzed by a ^{1}H-ENDOR simulation program, which allows to compare computed spectra with the ENDOR spectra of the copper complex.

P-683

Structural studies of antigenic determinants in view of developing a vaccine against shigellosis

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Development of a vaccine against human dysentery caused by *Shigella,* an invasive enterobacterium, is one of the priorities of the WHO. Four species of *Shigella* possess many serotypes defined by the structure of the O antigen, the main target of the protective immune response. They differ mainly by the position of the glucosyl residue (E).
Interactions of protective antibodies (Abs) with pentasaccharides of *Shigella flexneri* 5a and 2a repeating unit, and with their peptide mimics were investigated by NMR and molecular modeling.
Once the structures of ligands' free form were determined, we investigated its bound form using transferred NOE experiments. Both conformations were alike. Similarly, free peptide and that bound to Abs adopt a turn conformation.
Saturation transfer difference (STD) experiments were used to map the epitopes of the ligands in contact with Abs. Finally, NMR derived pentasccharide and peptide structures coupled to STD information allowed to generate Ab-pentasaccharide and Ab-peptide complexes.
The pentasaccharide glucose residue and the side chains of the peptide turn residues remain respectively in close contacts with the Ab and represent the ligand epitopes.

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P-684

The effects of modulation magnetic field on the bioelectrical parameters of streptozotocin-induced diabetic rat diaphragm muscles

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The aim of this study was to determine the effects of modulation magnetic field on the electrophysiological characteristics of diaphragm preparations in four groups. Intracellular recordings were performed in vitro in diaphragm fibers from streptozotocin -induced diabetic (D), non-diabetic Wistar rats (C), non-diabetic rats of exposed to modulation magnetic field (MMF) (CMF) and diabetic rats exposed to MMF (DMF). Muscle preparations were taken from the ventral-costal diaphragm muscle of the rats killed by decapitation. Comparing control to the other three groups muscle properties, resting membrane potential was significantly depolarised and action potential significantly decreased except diabetic rats exposed to MMF. The results of DMF haven't been evaluated yet. In this regard our studies are caring on.

P-684-B

Prediction of congestive heart failure by fractal analysis

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A number of new methods like fractal analysis have been recently developed to quantity complex heart rate dynamics. This method of analysis may reveal abnormalities in time-series data and the break down of fractal properties that are not apparent when the conventional statistical analysis methods are used. Heart Rate Variability (HRV) is a physiological parameter defined as the variation in the normal-to-normal RR interval during normal sinus rhythm. The measurement of HRV is non-invasive, often reproducible and rather easy to perform, which has lead to the popularity of HRV analysis. It is considered as an elegant method for the measurement of neuro-autonomic control of heartbeats as an autonomic cardiovascular regulation which is impaired in many clinical situations, such as hypertension, diabetes and coronary artery diseases. The law governing the evolution of long-term correlations in the RR series that reflects its fractal properties has shown by fractal analysis.
The present study relies on the reveals of fractal properties by using Detrended Fluctuation Analysis (DFA) applied on cardiac rhythm. It will be shown that DFA function F(n) of different RR series is approximated by power laws and the differences are observed between α-DFA coefficients (α1- α2) for healthy and diseased persons.
Former studies (Peng C.K.et al, 1993.1996) have shown that patients suffering from congestive heart failure have particularly low values of α2. Fractal analysis for healthy subject and patient suffering from CHF shows almost perfect power-law scaling with α2=1.05, while for the CHF data set, it was shown a break down of fractal dimension α2 to about 0.45. The obtained results indicate that elderly (68-81 years old), young (21-34 years old) have α2=0.43 $\pm$ 0.039, 0.48 $\pm$ 0.022 respectively.

P-685

Study of Serum Level of Some Trace Elements and Heavy Metals in Non Hodgkin´s Lymphoma

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Cancer is frequently associated with a combination of metabolic abnormality leading to a complex abnormal biochemical state in the tumor bearing host including alteration in vitamin and mineral concentration and consequently oxidant-antioxidant balance. Moreover the tumoricidal action of several anticancer drugs is known to be mediated by a free radicals dependant mechanism. It is the main mechanism of action of anticancer properties of some trace element. The study aimed to outline changes in serum level of some trace elements as Se, Zn, Cu and Mg. Also blood level of some heavy metals as Hg and Pb in non-Hodgkin's lymphoma patients before treatment and six months after successful treatments.
These elements are measured by EDXRF in all cases, as well as some radiological investigation, also histopathology from lymph node biopsy. A highly significant low mean serum level of Se, Zn and Mg and a highly significant high serum Cu level were elicited in NHL patients before treatment compared to the control group with $p < 0.01$. Follow up of NHL patients at remission showed that there is highly significant increase in the serum levels of Se, Zn, Mg and significant decrease in serum Cu level.

P-686

Multidrug resistance of tumor cell line P388 resistant to vincristine changes with tumor progression

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Multidrug resistance (MDR) is a significant obstacle to providing chemotherapy to many patients. One of the major reasons of its development is the overexpression of transport proteins, which exclude drug agents out of the cell. One of the promising strategies to overcome MDR would be to down-regulate the expression of MDR genes. Recently, it has been pointed out that expression of MDR transporters may be regulated by reactive oxygen species (ROS). We studied the resistance of leukemia P388 cells and P388 VR (vincristine resistant) on different days of growth and examined if there is any connection with alternation in oxygen concentration. Multidrug resistance was assessed spectrophotometrically using the change of calcein formation rate in cells from calcein ether in the presence of MDR inhibitor cyclosporin A. We showed that the rate of calcein formation differs in leukemia P388 cells on different days of growth: P388 cells have the highest calcein formation rate and P388 VR cells on the 7th day of growth have the lowest one. The ratio of calcein formation with and without Cyclosporin A, which is a characterization of MDR, changes with the cellular growth: it reaches maximum on the 7th day of growth. We suppose that the possible reason for change of MDR of ascitic tumor growth could be the down-regulation of MDR genes by ROS. Oxygenation was measured in P388 and P388 VR cells, it was the highest on the 3 day and on the 7^{th} day it was the lowest one.

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The effect of residual hydrogen peroxide on crystallin lens rabbits

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The present study was designed to investigate the effect of hydrogen peroxide (H_2O_2), as disinfectant, at the concentration of the residual levels found in the soft contact lenses, on rabbit's crystallin lens.Sixteen NewZealand rabbits were used in the present work. The rabbits were classified into four groups (8 eyes each). The first group (group I) was used as control. In the other three groups (groups II, III, and VI) 6 drops of H_2O_2 solution were instilled daily in their eyes with a concentration of 50 ppm, 150 ppm and 300 ppm respectively. After one month, the rabbits were decapitated and the activity of Na^+-K^+ ATPase was measured in lens membranes. The soluble lens proteins were studied using different techniques; and the enzymatic system of the lens was monitored. The results indicated a change in the molecular weight of different lens crystallins accompained with change of protein backbone structure after instillation of rabbit's eye with H_2O_2. Moreover, all the enzymes of the lens and lens membrane significantly changed in all H_2O_2 treated groups compared to control except for Na^+-K^+ ATPase after instillation of 50 ppm, which illustrate a non significant change. Thus, H_2O_2 at the concentration of the residual levels found in contact lenses after disinfection is hazardous and may lead to cataract.

P-688

Antioxidant reduces free radical mediated injury in tear fluid after photorefractive keratectomy

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The ability of antioxidants to scavenge reactive oxygen species is important to protect tissues from oxidative damage. The present work aimed to study the effectiveness of superoxide dismutase (SOD) in reducing tear fluid changes after photorefractive keratectomy (PRK).
Twenty-one Chinchilla rabbits were used in this study. Seven rabbits were served as control. Fourteen rabbits were divided into two groups and submitted to PRK with and without the use of (SOD). Tear samples were collected from the control and the two treated groups at intervals of 24 hours, one, two, three and four weeks after PRK respectively and the following studies were carried out: Molecular weight measurement; sFas and sFasL determinations; measurement of total proteins, ascorbic acid, cholesterol and phospholipids levels.
The results indicated changes in tears protein, lysozyme, sFas, and sFasL accompanied with decrease in ascorbic acid, cholesterol, and phospholipids levels after PRK. When the SOD was applied intraoperative and continues twice daily, the observed changes in tears fluid markedly reduced. However, further studies are needed to find the optimal concentrations and combinations of free radical scavengers (antioxidants) to be used during and / or after photorefractive keratectomy.

P-689

Biophysical characterization and bioactivity of divalent salt forms of bacterial lipopolysaccharide

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It is reported on a profound physicochemical characterization of rough mutant lipopolysaccharides Re (LPS Re) as divalent cation salt form or as natural salt form under the influence of externally added divalent cations. The investigations comprise DSC and FT-IR measurements of the $\beta \leftrightarrow \alpha$ gel to liquid crystalline phase behaviour of the acyl chains of LPS as well as synchrotron radiation X-ray diffraction measurements of their aggregate structures, and the calculation of the electron densities of the LPS bilayer systems. Furthermore, also the LPS-induced cytokine (interleukin-6) production in human mononuclear cells was determined. The results exhibit partially completely changed physicochemical parameters like acyl chain mobility and aggregate structures as compared to the natural or monovalent cation salt form. Importantly, also the biological activity exhibit considerable changes in particular for the Ca- and Ba-salts. The decrease in activity results mainly from the conversion of the unilamellar/cubic of LPS into a multilamellar structure.

P-690

Nucleation-controlled polymerization and pattern formation by polyalanine peptides

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Polyalanine expansions in the protein PABP2 induce misfolding and aggregation of the protein into insoluble deposits in muscle tissues and cell nuclei, causing oculopharyngeal muscular dystrophy (OPMD). To elucidate the mechanism of formation of disease-causing fibrillar protein deposits we have studied the aggregation properties of three synthetic peptides, containing seven (7-ala), eleven (11-ala) and seventeen (17-ala) successive alanine residues, each mimicking the N-terminal segment of PABP2 containing the polyalanine sequence. The effects of pH and alanine repeat length on secondary and higher-order structure of these peptides was investigated using CD, fluorescence and microscopy. At high pH both 11-ala and 17-ala (but **not** 7-ala) adopt secondary structures with high degree of β-sheet content and transform into fibrils after incubation for several weeks at room temperature. The time course of fibril formation shows evidence of a threshold incubation period (**delay time**), which decreases with increasing number of alanine repeats. Peptide solutions incubated longer than the delay time, when dried on glass slides form self-similar fractal patterns consisting of aggregates of individual fibrils. The fractal dimension of the patterns increases with incubation period and the number of alanine repeats, indicating a nucleation-controlled polymerization mechanism of formation of the aggregates.

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Dendritic spine increase in AD model mice at 4 months

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by progressive memory and cognition impairment. Synaptic damage and loss are believed to be responsible to the impairment. Here, dendritic spine density of granular cells in hippocampus of mutant human APP, PS1 and APP/PS1 double transgenic mice were measured and compared to Wild Type (WT) littermate controls by using confocal microscopy. No significant difference in spine density was observed between APP transgenic and WT mice at 4 months of age. However, significant increase in spine density was found in APP/PS1 double transgenic and PS1 transgenic mice as compared with WT controls. The spine density increase in APP/PS1 mice may be contributed to the PS1 overexpression or the effect of self-protecting response against the early A-beta toxicity.
[This work was supported by Tsinghua-Yue-Yuen Medical Sciences Fund.]

P-692

Characterization of bilayer membranes and peptide-membrane interactions by inner field compensation

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Planar membranes prepared according to the Montal-Mueller technique are a powerful tool to characterize peptide-membrane interactions. In particular, the determination of peptide-induced changes of the innermembrane potential difference is highly important. The interaction of cationic peptides, e.g. human antimicrobial peptide LL37, with membranes induces changes in its charge distribution and with that in the membrane potential profile. In the present study, we established time-resolved measurements of the capacitance minimization potential $\Delta\Psi$ on various asymmetric planar membranes made from phospholipids and lipopolysaccharides (LPS) – a major component of the outer leaflet of the outer membrane (OM) of Gram-negative bacteria – using the inner field compensation method. The results are compared to those of the innermembrane potential differences $\Delta\Phi$ determined from ion carrier transport measurements. We found, that the established model by Schoch et al. is well suited for phospholipid membranes, but not for the more complex architecture of reconstituted OMs. Thus, we expanded the model by considering an extended region of the LPS headgroups. The time courses of $\Delta\Psi$ have been used to characterize the interaction of LL37-derived peptides with reconstituted OMs. (Hagge et al., *Biophys J*, 2004)

P-693

Structure-activity relationship between target lipids and the antimicrobial peptide PMB

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For Gram-negative bacteria, the first target of interaction for antimicrobial peptides is the outer leaflet of their outer membrane which is composed mainly of the anionic lipopolysaccharide (LPS). Thus, the first step of the antimicrobial activity of the cationic peptide polymyxin B (PMB) against Gram-negative bacteria is electrostatically driven. After binding to LPS, PMB intercalates into the membrane and induces lesion formation, thus allowing further PMB molecules to permeate and to reach their final locus of action. We investigated the influence of the glycostructure of LPS on the interaction with PMB. To this end, various strains of *Salmonella enterica* serovar Minnesota differing in their LPS structure were used. The antimicrobial activity was determined by cell culture methods. As a measure for the surface potential, the ζ potential was determined for whole bacteria and for aggregates of the corresponding isolated LPS in dependence on the PMB concentration. Furthermore, the incorporation of PMB into LPS-monolayers was determined by film balance measurements, and the lesion-forming capacity was investigated using asymmetric planar bilayers. Our data allow to explain the different biological activities of PMB against different bacterial strains on the basis of their different glycostructures.

P-694

The effect of magnetic field on the symptoms in patient with irritable bowel syndrome

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Irritable bowel disease (IBS) is a syndrome that has no definite therapy. The nerve impulses travel to the brain through the spinal cord carrying the impulses of pain to the higher centers.
The idea of this work was to put a Magnet with a certain power at the back of the body above the level of the lumbar vertebrae, in order to interfere with the pain impulse pathway in an attempt to alleviate the symptoms.
Material of this work consisted of 22 volunteer patients with IBS based on Rome criteria (Thompson et al 1992). A small magnet of horseshoe shape (power =350 Gauss) was fixed by a belt on the back of the patient. They are adherent to the skin at the level of the 4^{th} thoracic vertebra. They are fixed during the day and removed at night to prevent irritation to patients. After one week the result was assessed and the symptoms were recorded and analyzed.
Result: improvement was assessed by suggested score all symptoms all the day = 5,about 50%=4,25%= 3, slight improvement =2, more than 3 days per week =1 and less than or no improvement = 0. Five patients had score 4, three had score 3, four had score 2, one had score 1 and 9 had score 0.
Conclusion: magnetic field interferes with pain pathway impulses. This could be used in the treatment of IBS syndrome. Further study is needed with different magnetic power and the use of magnetic pulsar instead of usual magnet may be of value.

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P-695

Fluorescence Spectroscopic Study of Hypericin-photosensitized Oxidation of Low-density Lipoproteins

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Low-density lipoproteins (LDL) play a key role in the delivery of hydrophobic photosensitizers to tumor cells in photodynamic therapy (PDT). One of the most promising photosensitizer in PDT appears to be hypericin (Hyp). Hyp is a natural photosensitizing pigment which displays a virucidal activity as well as antiproliferative and cytotoxic effect on tumor cells.
We demonstrate that Hyp interacts non-specifically with the lipid fraction of LDL by means of spectroscopic analysis. The molar ratio of monomeric Hyp binding to non-oxidized LDL and oxidized LDL is 30:1. Increasing the Hyp concentration further leads to the formation of Hyp aggregates inside the LDL molecule. We do also demonstrate that photoactivated Hyp oxidizes LDL in a light dose and excitation wavelength dependent manner. The level of oxidation of LDL depends on the amount of Hyp inside the LDL molecule. The maximum of the photosensitized oxidation of the LDL by Hyp is achieved for a 30:1 molar ratio, which corresponds to the maximum concentration of monomeric form of Hyp in LDL. These observations are important in view of the possible application of Hyp in PDT.

P-696

Metabolic modulation of low power long wavelength laser irradiation effects, seen in human T cells

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The aim of our studies was to contribute to the understanding of molecular and cellular mechanisms involved in low power long wavelength laser irradiation effects. We used AlGaInP/GaAs lasers (685nm/25mW, and 830nm/55mW) to irradiate cell suspensions with total incident doses of therapeutic significance (dose densities up to 60kJ/m^2), and monitored changes induced in cell membrane properties, intracellular ion homeostasis, and cellular signaling leading to cell survival/proliferation or apoptosis/necrosis. Exposing T leukemia lymphoblasts (Jurkat) and human peripheral blood lymphocytes to stress of various intensity and duration, caused by serum starvation, glucose deprivation, blockade of glycolysis, and/or blockade of oxidative phosphorylation, we compare short and long term laser irradiation effects seen in metabolically impaired and non-impaired cells. Our data document a cell type-dependent sensitive metabolic modulation both of membrane effects and calcium level changes manifest during/immediately after irradiation and of proliferation rates and deathstyle choices, apparent after hours/days.
Partial finantial support of the Romanian Ministry of Education and Research (CERES 264/2003, Grant 3-43) is gratefully acknowledged.

P-697

The effects of transcutaneous electrical nerve stimulation (TENS) on diabetic foot : a case report

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It is known that vascular problems, causing ischemic pain may occur in the lower extremities of diabetic patien and foot ulcers may develop as a result of the pathogenetic implication of peripheripheral neuropathy and insufficient circulation. Is she was case report presents a fifty year old female patient, having diabetes for 16 years she was admitted to Yuzuncu Yil University Research hospital, Internal Medicine Outpatients Clinics with complaints of pain resulting from diabetic polyneuropathy and ulcers in his feet. Foot ulcers of the patient gradually improved during TENS sessions and recovered by the and of the 30 days treatment. Studies on vasodilatation effects of TENS on such patients and the role of TENS treatment in the healing of chronic ulcers were discussed.

P-698

Development of a selection method for peptide aptamers inhibitory to proteases

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RNA/DNA aptamers have been developed for the various purposes: inhibitors, ribozymes, affinity-labeling reagents and others. However, peptide aptamers are not yet developed due to the unavailability of effective methods. Namely, though the *in vitro* selection/evolution technologies require the step of amplification of molecules, peptides can not be amplified directly. This situation was overcome by the introduction of a sophisticated method termed *in vitro virus* (IVV), which was built up from the coding part (RNA or DNA) and its product part (peptide or protein) with both covalently linked by a spacer molecule (Nemoto et al., *FEBS Lett*. (1997)) since the coding part of DNA/RNA allows us to amplify it. In addition, by introducing novel technologies for effective selection of peptide aptamers (i.e., Y-ligation-based block shuffling (YLBS) (Kitamura et al. *Protein Eng.* (2002))and SF link method (to be submitted)), we have completed a basic protocol for this purpose and applied this to pan peptide molecules having a function of protease-inhibition. Here, we report the method established for selecting peptide aptamers, comparing the effectiveness of this method with that developed for DNA aptamer selection.

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P-699

Evaluation of lead and cadmium levels in saphenous veins of smoking and non-smoking patients treated with coronary artery bypass grafting (cabg)

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The objective of this study was to assess cadmium and lead in samples of redundant saphenous vein (SV) obtained from 52 patients during coronary artery bypass grafting. There were 33 men and 19 women in the studied group, among them there were 31 active smokers and 21 patients with non-smoking history. Lead and cadmium levels in SV samples were determined by atomic absorption spectrometry.
The cadmium content (mean ± SEM) was higher in the smokers group (0,5667±0,05538 μg/g dry wt) than in the non-smokers group (0,1369±0,02858 μg/g dry wt, Mann Whitney test, P<0,0001).
The lead content (mean ± SEM) was higher in the smokers group (27,025±2,702μg/g dry wt) than in the non-smokers group (6,237±1,321 μg/g dry wt, Mann Whitney test, P<0,0001). Obtained results show cadmium and lead significant accumulation in smokers SV.

P-700

In vitro photodynamic treatment by phthalocyanine sensitizer on cancer cell lines

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Photodynamic therapy (PDT) is a promising method for the treatment of tumours. The photochemical interactions of sensitizer, light and molecular oxygen produce singlet oxygen and other forms of active oxygen, such as peroxide, hydroxyl radical and superoxide ion. The resulting damage of organelles within malignant cells leads to tumor ablation. The promising second generation of sensitizers - phthalocyanine $ClAlPcS_2$, was tested as an inducer of the photodamage. We report the cellular uptake, the production of reactive oxygen species (ROS) and the phototoxicity of $ClAlPcS_2$ on MCF7 human breast adenocarcinoma cells. As a source of radiation a semiconductor laser (λ = 675 nm) was used. Viability of cells was determined by means of molecular probes for fluorescence microscopy (calcein AM, ethidium homodimer). The quantitative cell viability changes in relation to sensitizer concentration and irradiation doses were proved by fluoroscan. ROS generation and H_2O_2 release after PDT on MCF7 cells were detected using probe CM-H_2DCFDA and recorded by luminescence spectrometer. Viability studies shown that the optimum phototoxic effect tested on MCF7 carcinoma cells was determined in the combination of laser dose 10 J/cm^2 and concentration of $ClAlPcS_2$ 2μg/ml. This work was supported by the grant No.MSM 6198959216.

P-701

Study of laser light penetration into the tissue

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Laser radiation has been now frequently used in medicine. With regard to the importance of having accurate information about the optical parameters of the tissue for laser therapy and for phototherapy in general, the aim of the paper was to verify these parameters. In this study, He-Ne laser, (λ = 632.8 nm), semiconductor laser (λ = 675.8 nm) and Argon laser (λ = 488 nm) were used. Distribution of the irradiance of laser radiation was detected by CCD camera and evaluated by image analysis software DIPS. The skin samples were obtained from plastic surgery. Laser radiation of wavelength 488 nm, 632.8 nm and 675 nm penetrates into all skin layers. The thickest specimen was (19 mm) and about 0.2 % of Argon laser light, 0.3% of He-Ne laser light and 2.1 % of semiconductor laser light penetrated this sample. The obtained results demonstrate the percentage of incident light penetrating the individual skin layers in various localization on skin surface which is a decisive factor for selection of radiation dose. This work was supported by the grant project of Ministry of Education MSM 6198959216.

P-702

In vitro influnce of some local anaesthetics on the surface electrical properties of erythrocytes

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Four molecules (Procaine, Tetracaine, Bupivacaine and Lidocaine) of either the ester or amide type of local anesthetics were studied. Erythrocytes were used to provide the membrane model. The surface electrical properties were determined by the electro-rotation technique. It was found that the surface conductance, K_s was the most sensitive parameter, while the membrane capacity (C_M) refractory to treatment with the local anesthetics tested. The values for K_s decreased with the increase in the concentrations of the local anesthetics giving concentration-response relationships. The values of the EC_{50} were found to be in the following order: Tetracaine (22.63 nM) > Bupivacaine (45.91) > procaine (59.57 nM) = Lidocaine (59.73 nM). The values were inversely proportional with lipid/water partition coefficients of the respective molecules.

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P-703

Investigating electrical properties of splenic lymphocytes of bilharzial, nonbilharzial subjects

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The membrane surface electrical properties of spleinc lymphocytes from an accident victim (control) and from Egyptian bilharzial patients were studied by electro-rotation. Phytohaemagglutenines (PHA-P and PHA-M) or pokeweed (PWM) were the mitogens used for cell stimulation. The unstimulated bilharzial lymphocytes had larger radii than those from the control probably because they were already stimulated by the disease state. After stimulation, all lymphocytes increased steadily in size and consequently in membrane area. The values for membrane capacity (C_M) were higher for control lymphocytes than for bilharzial cells. All C_M values increased with the duration of stimulation except for the bilharzilal cells incubated with PMW, which peaked on day 1 and deteriorated very rapidly after that. The values calculated for membrane conductivity (G_M) followed a different pattern. The C_M, levels exhibited peak values after different durations of stimulations depending on the cell type (control or bilharzial) and the mitogen used. The changes in conductivity may reflect alterations in trans-membrane transfer activity.

P-704

Thermotolerance in human erythrocyte

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Mechanism leading to cell death following heat is still not understood. It has been suggested that plasma membrane can be an important target, and that it plays some role in thermotolerance. Our earlier experiments showed that enucleated erythrocytes become resistant to the second heat shock. Erythrocytes were incubated at 44°C (15 min) and then at 37°C (0,25 – 16 h) and at 48,5°C (30 min). We observed that thermotolerance of erythrocytes was transitional phenomen reaching its maximum at 3h of the incubation at 37°C between the heat shocks and disappeared after 6 hours. After preincubation at 44°C the activity of ATPase increased. Antioxidant compounds quercitin and baikalin when added to incubation medium can regulate the appearance the thermotoloerance. It has been hypothesised that hyperthermia promotes formation of free radicals in cells. In our experiments we examine formation of free radicals by fluorescence and EPR methods. We did not observe any difference between free radicals formatting during by one or two heat shocks. No lipid peroxidation in erythrocyte membrane was observed. Since flavonoids are inhibitors of activity of ATPase, it is possible that changes in activity of ATPase can influence the thermotolerance.

P-705

Activation of oxygen in monocytes of healthy children and with pollinosis

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ROS generation ability of monocytes in patients with pollinosis before and after SIT was analysed by chemiluminescence (CL) method. Monocytes were isolated from the blood of 15 children aged 9-17 years with pollinosis.Control group consisted of 10 healthy volunteers aged 9-17 years. ROS production in monocytes activated during adhesion to glass was significantly higher in pollinosis children before SIT than in norm. Application of SIT was accompanied by decrease of monocyte ability to produce ROS during adhesion to glass, but did not reach the values obtained for control group. Inhibition of $PLA_2$by indomethacindecreased summary yield of ROS in monocytes of patients in 2 times and in control group in 4 times. MK-886, aspirin, LY294002, PD98059, Go6983 influence on ROS generation in monocytes of patients in the same way as in healthy people. H_2O_2 did not influence on adhesion-associated ROS formation in monocytes from healthy and allergic subjects. However, H_2O_2induced the significant increase of monocyte response to fMLP in control specimens, but in patients H_2O_2 led to the decrease of fMLP-induced CL of monocytes. It was shown that H_2O_2 induced increase $[Ca^{2+}]$ by $1{,}6\cdot10^{-8}$mol/l in monocytes of patients and by $3{,}2\cdot10^{-8}$mol/l in monocytes of healthy people. So monocytes from pollinosis children demonstrate abnormal response to stimulation with H_2O_2 and fMLP.

P-706

3D ^{1}H MR spectroscopic imaging and diffusion weighted imaging of prostate in men with raised PSA

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The utility of 3D ^{1}H MR spectroscopic imaging (MRSI) and diffusion weighted imaging (DWI) at 1.5T was evaluated in men with PSA 4 to 20 ng/mL. Subjects (n = 28) were divided in to two groups (Group I – n = 9, age = 68.3 $\pm$ 11.35 yrs, PSA > 20 ng/mL and Group II - PSA = 4 – 20 ng/mL, n = 19, age = 60.21 $\pm$ 15.1 yrs). Metabolite [Cit/(Cho+Cr)] ratio and apparent diffusion coefficient (ADC) values were calculated from the same ROI where MRSI showed decreased [Cit/(Cho+Cr)].
In Group I, the [Cit/(Cho+Cr)] ratio was < 1.4 in PZ the corresponding ADC values were in the range 0.6 to 0.7 mm^2/s. Interestingly, the [Cit/(Cho+Cr)] ratio was < 0.7 for different locations in PZ of subjects Group II. ADC for the identical locations showed values ($1.00 \pm 0.22 \times 10^{-3}$ mm^2/s) less than observed for normal PZ tissue ($1.7 \pm 0.25 \times 10^{-3}$ mm^2/s).
Reduced metabolite ratio and ADC observed for Group I is due to altered metabolism and tissue structure. In subjects of Group II, observation of lowered [Cit/(Cho+Cr)] ratio and ADC compared to normal tissues in certain areas indicate changes associated with malignancy. Results suggest that the combined use of MRSI and DWI may have greater utility for diagnosis of prostate cancer in the patients whose PSA is 4 - 20 ng/mL.

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3D ^{1}H MRSI directed TRUS-guided biopsy for detecting prostate cancer in men with PSA 4-10 ng/mL

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We evaluated the success rate of 3D proton MRSI targeted TRUS guided biopsy (Group I) compared to the patients who had TRUS guided sextant biopsy without MRSI (Group II) in the detection of cancer foci in men with PSA range 4 – 10 ng/mL (n = 25, age = 60.5 $\pm$ 6.5 years.). The investigations were carried out at 1.5T. Metabolite ratio [Cho+Cr)/Cit] < 0.7 was considered as normal, 0.7 – 0.85 as suspicious, and > 0.86 as malignant. TRUS guided biopsy were carried out in Group I patients who showed suspicious area of malignancy in MRSI.
5/25 patients (20%) of Group I showed positive for malignancy on histology as compared to 12/123 patients (9.75%) who underwent TRUS-guided biopsy without prior MRSI. 15/25 patients showed voxel suspicious of malignancy. None of the patients who had a negative MRSI were found to have prostate cancer on histopathology.
These preliminary results reveal that the detection rate of prostate cancer in men with PSA level 4 – 10 ng/mL, increases if TRUS guided biopsy is performed using the coordinates of the suspicious site derived from MRSI data.

P-708

Insertion of amphitropic proteins into membrane models: threshold effect

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In biology, interaction of water-soluble molecules (proteins, chemotherapeutic drugs…) with phospholipid membranes is an interfacial process of crucial interest, like in haemostasis, atherosclerosis or cancer. Since the function of amphitropic proteins depends on their spatial organisation relative to the membrane leaflet to which they bind, it is fundamental to be able to study how they bind to a phospholipid layer. For this purpose, it is necessary to use membrane models; the validity of two of them was tested on a biological system (one macromolecular complex in coagulation cascade) and gave consistent (comparable) results. Complementary methods of direct surface measurements on condensed monolayers (electrochemistry and radioactivity) were used, in association with radiolabeled photochemistry on vesicles. Ac polarography allows distinction between properties (stability) of different types of lipid layers and mainly unambiguously between adsorption and insertion of water-soluble molecules. Thus, it was found that they insert at a specific surface concentration threshold, depending on the type of lipids and proteins (apolipoprotein, proenzyme, enzyme, cofactor…) involved in the process. The structural domains of the whole proteins imbedded in the membrane could be detected by photolabeling, and they correspond to the purified fragments which insert in the monolayer. Moreover, voltammetry and radioactivity gave quantitative informations.

P-709

Influences of electromagnetic irradiation of high-voltage transmission lines on procreate organs of animals

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The influences of electromagnetic field(EMF) of high-voltage transmission line(HVTL) on properties of procreate organs for rats are studied. The rats in 4^{th} weeks were divided into controlled and exposed groups. The latter is exposed in EMF about 400days. The EMF of HVTL is generated by an appliance. Their strengths of the electric and magnetic fields are 4000V/m and 0.09-0.1G, respectively, which are the strengths of the electric and magnetic fields at the position of 2.0-2.3 m distancing the earth under the HVTL of 220KV. This strength of the EMF is measured by us in surrounding of HVTL of 220KV. The spermary and ovary in the procreate systems are extracted from killed rates. Their shapes and structures are observed by optic and electric microscopicmeter. We discover that the shapes and structures of the cells in spermary tissues are varied, expansion of mitochondria and increase of density in side of chromosome, under the influences of the EMF of HVTL. We measure also the spectra of infrared absorption of the spermary and ovary by Nicolet FT-IR 670 spectrometer and found that the spectra of the controlled and exposed groups are different. Very surprisingly, the new and strong band of 2345 cm^{-1} occurs simultaneously in the spermary and ovary. This shows clearly that structure or conformation of the molecules in the spermary and ovary under influence of the EMF of HVTL, which results just in above changes of the spermary and ovary in the procreate systems of animals.

P-710

^{14}N and ^{31}P MAS NMR: new tools to measure electrostatically driven action of membrane surface-active proteins/peptides

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Exploiting naturally abundant ^{14}N and ^{31}P nuclei by high-resolution MAS NMR provides a molecular view of the electrostatic potential present at the surface of biological model membranes, the electrostatic charge distribution across the membrane interface, and changes that occur upon electrostatically triggered action of surface active proteins/peptides[1-2].^{31}P and ^{14}N MAS-NMR probe directly the negatively charged phosphate and positively charged choline segment of the electrostatic P^{-}-O-CH_2-CH_2-$N^{+}(CH_3)_3$ headgroup dipole of zwitterionic DMPC in mixed lipid systems. The isotropic shifts report directly on the size of the potential existing at the phosphate and ammonium group whilst the anisotropic chemical shielding (^{31}P) and quadrupolar interaction (^{14}N) characterize changes in headgroup orientation in response to surface potential. In the case of the neuropeptide nociceptin, a diffusive ligand involved in GPCR signal transduction, the observed changes in the NMR spectra supported the hypothesis that membrane association is an important prerequisite to the binding of nociceptin on the ORL1 GPCR receptor [2].
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P-711

The interaction of peptide antibiotics with model membranes investigated by SAXS measurements

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Nowadays a severe problem in the therapy of bacterial and fungal infections with classical antibiotics is the uprising resistance of pathogenic bacteria against these treatments. Therefore an immense interest to find alternative antimicrobial substances developed. Beside others, one class of promising molecules are short antimicrobial peptides that belong to the innate immune system of many different species and also derivatives thereof. Their mechanism of action differs from that of conventional antibiotics, because these peptides show a direct interaction with the membranes of their target cells. Peptide antibiotics show a high specificity for bacteria and fungi over mammalian cells, but so far the exact mode of action and the cause for their selectivity is not fully understood.
In our work we first tested the activity of the antimicrobial peptide NK-CS and different modifications thereof against *E. coli* bacterial cultures and also their haemolytic properties against human red blood cells to get information about their selectivity. Subsequently we used small angle X-ray scattering to investigate the interaction of the peptides with model membranes and observed an influence on the inverse hexagonal phase transition of lipids with phosphatidylethanolamine head groups. The influence of the peptides shifts the phase transition temperature and promotes a curvature of the membranes. This curvature could finally lead to the disruption of the model membranes.

P-712

The effect of electromagnetic field irradiated by high-voltage transmission lines on the proliferation of embryo fibroblast

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Proliferation states of chick embryo fibroblast cells in vitro under the action of electro-magnetic field irradiating by high-voltage transmission lines have been investigated by 3-(4,5- dimethylthiazold 2-yl)-2,5 diphenyltetrazolium bromide (MTT) technique. The chick embryo fibroblast (CEF) cells were divided into controlled and exposed groups, the experimental group is exposed in this electromagnetic radiation of high-voltage transmis- sion. The electromagnetic field is generated by an appliance. The strengths of their elect- ric and magnetic fields are 4000 V/m and 0.09-0.1 G, respectively, which are the strengths of the electric and magnetic fields at the position of 2.0-2.3 m distancing the earth under the high-voltage transmission lines of 220KV. This strength of the electromagnetic field was concretely measured and obtained by us in surrounding of the high-voltage transmis- sion lines of 220 KV. The results obtained show that the proliferation of CEF was inhibited by this electromagnetic field, the ratio of proliferation of CEF descends with the increase of the time of irradiation, the number of the CEF cell decreases for the exposed group when compared with that of the controlled group. This shows that the electromagnetic field of the high–voltage transmission line can suppress the proliferation of CEF in vitro.

P-713

Tyrosine-containing peptides as indicator of endogenous intoxication in patients with cancer

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The increase of total content of peptides in a blood is one of widely used markers of endogenous intoxication of an organism. The total content is measured on the absorption of protein-absent plasma at 254 or 280 nm. However, this test does not reply on early stages of endogenous intoxication. We propose to use the concentration of tyrosine-contained peptides in a blood plasma as a new marker for intoxication. Our aim was to evaluate whether tyrosine-containing peptides measurement might provide the better diagnostics of endogeneous intoxication than total content of peptides. 30 patients with cancer of ovary and 45 patients with cancer of lung were examined. 40 healthy people were a referent group. The tyrosine-containing peptides content was estimated by our technique and the total peptide content was measured by absorbance of plasma after protein removal at 254 nm. The patients with cancer of ovary had the increase of tyrosine-containing peptides content and total peptide content of 401% and 180%, respectively, in comparison with the referent group. In case of cancer of lung, these parameters were 409% (tyrosine-containing peptides) and 170% (total) in comparison with the referent group. These results suggest that the tyrosine-containing peptides content in plasma is more sensitive marker than the total peptide content for diagnostics of endogenous intoxication syndrome and their monitoring at different cancers.

P-714

Lipidic membranes are potential "catalysts" in ligand activity of the pentapeptide neokyotorphin

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NKT is a multifunctional pentapeptide, involved in biological functions as diverse as analgesia, antihibernatic regulation and proliferation stimulus of tumour cells. The interaction of NKT with cell membranes is potentially important to all these multiple processes, since receptor-mediated processes were proposed. Sargent and Schwyzer proposed in their “membrane catalysis” model that ligands would interact with membrane lipids in order to adopt the necessary conformation for cell receptors. We have used fluorescence techniques to study in-depth location, orientation and extent of incorporation of NKT in model systems of membranes. The roles of lipid charge, membrane phase and sterol presence were investigated. The phenolic ring of tyrosine is located in a shallow position in membranes. The extent of partition decreases in gel crystalline membranes relative to liquid crystalline membranes. Addition of cholesterol causes a reorientation of the tyrosine ring in the interface of lipidic bilayers. Lipidic membranes meet all the conditions to be potential “catalysts” in the ligand activity of the multifunctional pentapeptide NKT because they modulate the exposure and orientation of the phenolic ring, which is most likely involved in docking to receptors.

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P-715

Study of cellular damage by comet assay after PDT *in vitro*

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Photodynamic therapy (PDT) is based on cytotoxic action of sensitizers that bound to cells and are excited by appropriate light source. PDT is the method of choice in treatment of some superficial tumours. The goal of project was study of cytotoxic effect of new generation of sensitizers alone or bound to cyclodextrin carrier (10 μM $ZnTPPS_4$ and 1 mM hpβCD) and analysis of DNA damage in the cell line of the human melanoma G361 after PDT by comet assay. It is a method for detecting DNA strand breaks at the level of single cells. Cells with DNA damage appear as fluorescent comets with tails of DNA fragmentation. Cells with undamaged DNA appear as round spots, because their intact DNA does not migrate out of the nucleus. DNA fragmentation was detected after 5, 10, 20, 40 and 80 minutes of irradiation. As a source of radiation was used violet LEDs (luminiscent diods emitted radiation). Cells in medium, cells in medium with $ZnTPPS_4$ and cells in medium without $ZnTPPS_4$ irradiated 80 minutes were used as controls. The optimal irradiation time is about 20 minutes. This work was supported by the grant project of Ministry of Education MSM No.6198959216.

P-716

Determination of the incorporation of 2,5-disubstituted-1,3,4-thiadiazole anti-tuberculosis agents into artificial bilayer-lipid membrane

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A series of alkyl α-(5-aryl-1,3,4-thiadiazole-2-ylthio) acetic acid esters were synthesized and evaluated for their in-vitro anti-tuberculosis activities in strain H(37) Rv. The anti-tuberculosis data indicated that methyl,ethyl, propyl, buthyl and benzyl esters were able to control anti-mycobacterium tuberculosis activity in vitro, significantly (MIC = 0.39-0.78 μg/ml). The hydrophobic tails of these agents seems to contribute to their interactions with membrane. Accordingly, using longer acetic acid esters as stating materials, we attached more hydrophobic side chains. However, because of the slow growth and complexity of membrane of *M. tuberculosis,* as well as the need for a simple investigation system, artificial membranes were used to evaluate the reconstitution process. Thus, using artificial planar bilayer we are trying to study the passage of these novel molecules through some membrane channel forming proteins, OmpF. The charged groups incorporated into the agent's structure acts as an anchoring point by which the agent is pulled into the channel. The details of this approach that might shed some light on the establishment of a more efficient way to control the *Mycobacterium tuberculosis* growth will be presented.

P-717

The contribution of aqueous humor in ArF 193 nm excimer laser photorefractive keratectomy

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Ultraviolet light (193 nm) produced by an excimer laser has been used to produce precise tissue ablation with minimal thermal damage to adjacent tissue. The present study was designed to investigate the effect of photorefractive keratectomy (PRK) procedure on aqueous humor constituents and, also the use of antioxidant enzyme superoxide dismutase (SOD) – applied topically – to interfere with these changes (if any). Five groups of schenchilla rabbits were involved in this study, where four groups underwent corneal stromal ablation using argon fluoride excimer laser (ArF, 193 nm). Two of these four groups were treated with superoxide dismutase intraoperativley. The resulted data were compared with control one, the fifth group. Aqueous humor refractive index, cholesterol, phospholipids, malondialdehayde (MDA) and total protein were measured and discussed properly. The obtained results revealed depletion of aqueous humor ascorbate and GSH content. In conclusion, PRK induce changes in aqueous humor constituents. These changes lasts at least for 24 hours and become subtle after 4 weeks. The use of exogenous SOD seems to exert beneficial effect on aqueous humor refractive index and total protein.

P-718

Role of membrane composition on the accessibility of amyloid precursor protein alpha-cleavage site

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The membrane composition has been shown to play an important role in the function of membrane proteins. This project aims at studying how changes in the brain lipid membrane composition in Alzheimer's disease may affect the processing of the amyloid precursor protein (APP). For this purpose we exploit ^2H and ^{15}N solid-state NMR. Comparison of the anisotropy observed in the ^2H spectra of a synthetic fragment of APP labelled in the transmembrane domain indicates the peptide adopts an alpha-helical conformation upon reconstitution into DMPC and DSPC vesicles. ^{15}N spectra of the ^{15}N labelled alanine in the vicinity of the alpha cleavage site, which is close to the putative transmembrane domain, showed a lipid-specific scaling of the chemical-shielding anisotropy. The influence of the lipid environment on the mobility observed at the ^{15}N labelled site in vesicles suggests that the accessibility of the site for proteolytic cleavage could be controlled by the characteristics of the lipid bilayer. These findings are important in view of APP processing: changes in the lipid environment could affect the accessibility of the α-cleavage site for the secretase and induce the amyloidogenic processing. Enzymatic cleavage assays with the APP fragment incorporated in vesicles will reveal more information.

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P-719

Interplay of molecular and biophysical mechanisms in tumor invasion

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Tumor invasion requires the acquisition of migratory capacity by the constituent cells, established through by the competition of cell-cell and cell-matrix interactions. To study the contribution of these factors, adhesive and invasive properties of nine human brain tumors were investigated, using molecular and biophysical approaches. The biomolecular analysis entailed to measuring the expression level of N-cadherin (predominant cell adhesion molecule in these cells), along with the mRNA level of proteins involved in matrix degradation (i.e. matrix metalloproteases and their inhibitors). Biophysically, cell adhesion strength was quantified in terms of surface tension, a physical parameter related to tissue cohesivity, as well as through the surface morphology of three-dimensional cell aggregates using scanning electron microscopy. Strict quantitative correlation between the measured quantities (N-cadherin level, tissue cohesivity. MMP level and invasive capacity) was observed. We discovered striking variation in the invasive patterns of the different tumors that can only be interpreted by the competition of the measured factors.

P-720

Interaction of three overlapping peptide sequences of E2-GBV-C/HGV virus protein with lipid bilayers

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Hepatitis G virus (GBV-C/HGV) is frequently associated with hepatitis C virus (HCV) infection. People co-infected with GBV-C/HGV and human immunodeficiency virus (HIV) have delayed progression of the HIV disease. The liver disease caused by GBV-C/HGV and the mechanism how this virus could inhibit the progression of AIDS needs to be defined. At this work, we report the effect of the three overlapping synthetic peptides of E2 protein of GBV-C/HGV: E2 (26-53), E2 (32-53) and E2 (39-53) on lipid bilayers by fluorescence techniques: resonance energy transfer (RET) and ANTS/DPX fusion assay. The complete peptide sequence is ^{25}GSRVPTGERVWDRGNVTLLCDCPNPWV^{54}. Small (SUVs) and large (LUVs) unilamellar vesicles prepared by sonication and extrusion respectively were used [composition: 1-palmitoyl-2-oleoylglycero-sn-3-phosphocholine-sphingomyelin-1,2-dipalmitoyl-phophatidyletanolamine-1,2-dipalmitoyl-phosphatidylserine (POPC-SM-DPPE-PS) (40:33:12:15)]. The vesicle mixture used for RET contained 0,6% of NBD-PE or Rh-PE co-dispersed with the unlabelled lipids to obtain the desired lipid composition. Excitation and emission fluorescence spectra from NBD were registered. The results show that the three overlapping sequences are able to cause destabilization of lipid membrane at the pH range from 7,4 to 4,0. This effect is greater as longer is the peptide sequence and the more acidic is the pH medium.

P-721

Ultraweak cell radiation and its relation to the early embryonic development

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In our research of ultraweak cell radiation and its relation to the early embryonic development inductors and detectors of this radiation are biological systems (eggs, embryos). Analyzing resultant effects of biophoton emission we have observed the following features:
- mortality on different stages of embryogenesis;
- occurrence of developmental anomalies;
- synchronization or desynchronization of development.
We carry out our study by means of histochemical methods and microscopy accessories. It allows us to investigate different structures (cells, tissues, organs) during developmental processes. We have also used some optical elements for control these interactions.

P-722

Electrical and motor oscillations of vesical detrusor and trigone

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Introduction: Normal and pathological *vesical motor function* leading to complicate diseases (incontinence, cystitits, pyelonephritis, hypertension) is not clarified.
Method [1, 2]: Isolated strips of guinea pig (human, rabbit) *detrusor* (D) and *trigone* (T) generate fast *phasic* (D: 2.9$\pm$0.5/min) and slow *tonic* (T: 0.3$\pm$0.1/min) motor *oscillations* (n=55).
Results: Recent and earlier results demonstrate a similar dependence of length from stretch of D and T, whereby differences in mechanical *"hysteresis"* (after recovery) under isometric (3 to 80 mN) and isotonic (0.5 to 3 g) conditions were observed. After stretch *contractile amplitudes* of D increased over 300%, but about 150% of T, without difference of *frequency*. *Spike* activity (D myocytes) was transformed into *burst-plateau* one after stretch (n=54).
Conclusion: D and T motor oscillations are probably related to electrical ones, generated by stretch-dependent ionic (Ca-activated K-) channels.
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P-723

Alpha1-acid glycoprotein as marker of different diseases

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Alph1-acid glycoprotein (AGP) is acute phase protein which concentration is rapidly increased in different diseases including wide spectra from tuberculosis to cancer. For diagnostics of these cases the clinicists need fast and not time-consumed test of determination of inflammatory markers in human blood. AGP consist of two parts: protein and carbohydrate chains, which form a glycan core in glycoprotein. We developed a fluorescent method of AGP determination in human blood using fluorescent probe Quinaldine Red which specifically binds with AGP on cationic binding site of protein part. Fluorescent measurements strongly correlate with IFA methods but IFA is more expansive and need additional setups in clinical laboratories. With close collaboration of Byelorusian Cancer Center we measured more then 50 samples of patient's plasma having breast and lung cancer and concluded that our method is high sensitive and adequate for all requirements. In case of breast cancer the concentration of AGP has increased ten-folds. In several patients we did not observe increasing of AGP level but we detected increasing of tryptophan fluorescence we explained as the conformational changes of AGP is following to the movement of Trp residues to the inner pocket of protein part.
Thus we should take in consideration as concentration of AGP as binding properties, which depend on conformational state of glycoprotein.

P-724

Enhancement of ultrasonically induced cell damage by phthalocyanines in vitro

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The study of ultrasound bioeffects and their mechanism is of fundamental importance. Biological effects of ultrasound are due to one or a combination several factors. One of them are free radicals, which are formed by pyrolysis of molecules present inside collapsing cavitation microbubbles (such as H• and •OH) and secondary radicals formed in reaction between hydrogen and dissolved molecules. In our study, we observed formation of ROS (reactive oxygen species) after sonication with 1 MHz continuous ultrasound at the intensities of 0.61-2.44 W/cm^2. The role of the ultrasound produced free radicals and other reactive oxygen species has been implicated in the mechanism of sonodynamic treatment of cancer (synergistic effects of chemicals and ultrasound). Furthermore, two phthalocyanines (zinc and chloroaluminum) have been tested as potential sonosensitizers for sonodynamic therapy. We investigated the effect of ultrasound and phthalocyanines on carp erythrocytes, as nucleated cell model. The erythrocytes were exposed to 1 MHz continuous wave at the intensity of 2.44 W/cm^2 in the presence or absence of phthalocyanines. The influence of phthalocyanines alone was also examined. Ultrasound and phthalocyanines simultaneous exposure led to an increase in the level of hemolysis, lipid peroxidation and osmotic fragility in comparison to ultrasound or phthalocyanines alone. These results suggest that phthalocyanines show sonosensitizing properties.

P-725

Influence of GSM electromagnetic field on fluidity of mononuclear blood cells membrane

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Introduction: The aim of this work is to determine if there is any influence on fluorescence anisotropy of human mononuclear blood cells by exposing to the GSM electromagnetic field.
Materials and Methods: Plasma membrane fluidity of the mononuclear blood cells separated from fresh peripheral blood with Ficoll-Hystopaque was assessed using the fluorescence depolarization of TMA-DPH. The irradiation parameters were: microwave frequency 1,800 MHz, irradiation time 30 min, power densities 48 mW/ cm^2 (SAR=18 mW/g, Specific Absorption Rate), temperature 36.5 – 37.5°C, in the following sequence: 30 min without irradiation, the next 30 min with GSM irradiation and 30 min after irradiation. We used 8 samples from one normal subject: 4 samples were exposed (E) and 4 samples were shamed exposed (SE) to GSM radiation.
Results and Discussions: The membrane anisotropy slowly decreases in time. For those 4 couples of cells, E and SE, we noticed that anisotropy of E cells decreases in time slower that anisotropy of SE cells. The differences between normalized anisotropy of E cells and SE cells are about 2.05%, (p=0.012) for the irradiation period and about 3.05% (p=0.024) after irradiation period.
Conclusions: Our results show that 30 min of exposure at 1,800 MHz microwaves, (SAR=18mW/g) influences mononuclear blood cells membrane fluidity.

P-726

Dielectrophoreic behavior of erythroleukemia cells using interdigital microelectrodes

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Dielectrophoresis is defined as the lateral motion impared or unchanged particles as a result of polarization induced by non-uniform electric field. It has been used to study cells and cellular alteration accompanying physiological changes.
The aim of this work to report on the electrokinetic behavior exhibited by human leukemia cells subjected to traveling electric field of frequencies ranging from 10 KHz to 1MHz using positive and negative Dielectrophoreic forces generated by microelectrodes of an integrated castelled design (Price et al 1988)
Material and methods: the dielectrophoreic behavior of erythroleukemic cells of ten patients with acute myeloid leukemia were compared with erythrocytes behavior of ten healthy subjects of the same age and sex.when non-uniform electric field is created between microelectrodes, cells will redistribute themselves around the electrodes. The formed pattern of the cells at various frequencies was recorded microscopically.
Results: at low frequencies both normal and erythroleukemic cells were moved away from the electrodes to the bay region forming triangular shape. By raising the frequency of the fields, up to 100 KHz normal erythrocytes collected at the tips of the electrode, while erythroleukemic cells change pattern at a frequency of 200 KHz to the pearl chain form.
Conclusion: this method can be used to separate and manipulate many types of bioparticles such as normal erythrocytes from erythroleukemic cells in these patients.

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P-727

Nerve regeneration in rat spinal cord, a biophysical approach

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Injury to spinal cord is devastating and is very difficult to be monitored at cellular and molecular level *in vivo*. There are several types of spinal cord injury (SCI) mainly involving contusion and compression injuries, which disrupt nerve membrane structure and function.
Here, we are trying to employ different factors effective enough to promote nerve membrane fusion in order to restore the physiochemical condition of cytoplasm and also to reconstruct microtubules networks. Changes in axonal conduction properties of spinal cord was studied after an experimental compression injury. The experiment is conducted on rat and in a sucrose gap chamber where the effects of specific lipids, known to be able to reinitiate lipid bilayer formation due to their particular phase transition characteristics, are tested. The membrane healing recovery is monitored electrophysiologically by means of the recovery of the amplitude of the compound action potential (CAP) produced. In this report we further evaluate the biophysical nature of the nerve conduction, the combined effects of PEGs (polyethanol glycol), effects of electromagnetic field believed to further establish the appropriate molecular arrangement.

P-728

A method for quantifying the insulin effect on ROS production by human neutrophils

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Neutrophils (PMNLs) play an important role in a variety of physiological and pathological processes (such as host defence, postischemic reperfusion damage). During phagocytosis of microbial intruders, neutrophils increase their oxygen consumption (the "respiratory burst") through the activity of an NADPH-oxidase that generates superoxid anion (O_2^-) and hydrogen peroxide (H_2O_2). The primary products (superoxide anion and hydrogen peroxide) generated by NADPH-oxidase are not sufficiently reactive to account for the bactericidal effects; however, these oxygen species give rise to yet other reactive oxygen species (ROS) that are strongly antimicrobial.
In our work, we investigated the effect of insulin on ROS production by normal human neutrophils. The neutrophils were stimulated with opsonized zymosan, fMLP (formyl-methionyl-leucyl-phenylalanine) and PMA (phorbol-myristate-acetate) and incubated for 30 minutes with different insulin concentrations (0-50 μU/ml). For measuring ROS production, we used luminol-amplified chemiluminescence assay. We observed that the ROS production depends on insulin concentration: higher and lower concentrations than the physiological ones decrease ROS production. This is indirect evidence that the insulin may modulate the ROS production by normal human neutrophils through insulin receptors.

P-729

Primary effect of influence of artificial and natural electromagnetic fields on live organism

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Despite the modern development of the theory of electromagnetic fields interaction both in Classical and Quantum Electrodynamics, a world-wide opinion exists that the theoretical explanations of influence of natural and artificial electromagnetic fields on live organisms is impossible.
The explanation of the mechanism of this influence is vitally important owing to the development of new electronic devices working in different frequency ranges.
It is shown that the application of newly developed procedure of shutting-on of interaction of charged particles with electromagnetic fields enables one to explain their influence of live tissue by origination of macroscopic polarization currents due to the joint action of electric and magnetic components of electromagnetic waves. The greatest currents originate in the case of resonance between the proper frequencies of the medium and external electromagnetic fields. Thus, the experiments to measure these polarization currents may give one the information about dangerous frequency ranges and they should be excluded during construction of all electronic devices.

P-730

The influence of S-palmitoylation on the surface properties of Lipids-protein monolayers

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SP-C is important for a dynamic adaptation of surface tension of alveoli during the breathing cycle. SP-C facilitates the respreading of films from multilayers that have been formed during the compression. We are interested in the role of S-palmitoylation for the formation of lipid-protein protrusions. In consideration of these fatty acid chains may act as an anchor between two bilayers, identified as protrusions.
Our experimental model systems are composed of phospholipids (DPPC/DPPG 80:20 mol %) with SP-C or non-palmitoylated SP-C (SP-C_{up}) or short SP-C (25 amino acid; SP-C_s). SP-C_{up} and SP-C_s is either used to investigate the role of S-palmitoylation. Film balance technique, fluorescence light microscopy (FLM) and scanning force microscopy (SFM) have been used to investigate biophysical properties and the topography of lipid-protein monolayers.
In the system SP-C_{up} is able to build up protrusion but with a higher amount of protein. Whereas the shorter length of protein but with palmitoylation (SP-C_s) needs less amount of protein compared with SP-C_{up} to complete lung surfactant protein action. It showed smaller LC/LE domains but in a same pattern of native in contrast of SP-C_{up}. It means to support the idea that both α-helical and palmitoylation parts of SP-C are important for a role in multilayer formation during the breathing cycle in pulmonary surfactant.

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P-732

Mössbauer spectroscopy of iron containing proteins during molecular diseases

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Iron containing proteins play vitally important role in biological systems. It is well known that several diseases, so called molecular diseases, are caused or accompanied by the synthesis of anomalous biomolecules or any other protein biosynthesis disturbance. Some pathological states of the body are caused by environmental factors that may effect on the biological molecules. The iron electronic structure may reflect some structural changes in iron containing proteins related to pathological processes. In this case Mössbauer spectroscopy can be used as the most sensitive tool to study the iron electronic structure. This review considers the results of Mössbauer spectroscopic studies of hemoglobin, ferritin and hemosiderin during several molecular diseases such as hemoglobinopathies, iron overload diseases, leukemia as well as hemoglobin during effect of radiation and chemicals. Mössbauer hyperfine parameters characterized variations of the iron electronic structure in proteins resulting from protein destruction or structural changes during pathological processes. These data may be useful for additional analysis of diseases at the molecular level.

P-733

Behavior of myosin light chains in hibernation: the clue to mechanism of human cardiomyopathies

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In norm, cardiac myosin contains specific isoforms of light chains (LC) in atria (ALC1, ALC2) and in ventricles (VLC1, VLC2). Earlier we registered the appearance of 30-70% ALC1 in human ventricles at early stages of dilated cardiomyopathy (DCM) but not at terminal stage. Functional sense of such a change of LC1 composition in DCM we tested using the hibernation of ground squirrels as a nature model of reversible suppression of heart function. In ventricles of awaking animals we revealed the appearance of ALC1 up to 30%. These isoform changes lead to the increase of actin-activated ATPase activity of myosin that promotes rapid recovery of heart function. In atria of hibernating squirrels we found 30-60% VLC1, not typical for atria of active animals. As enzymatic activity of atrial myosin higher than that of ventricular one, the replacement of ALC1 by VLC1 upon hibernation (like situation in tetralogy of Fallot) may be aimed at reversible decrease of contractile activity that is necessary in this period. Thus the comparative studies of hibernation and cardiac diseases help to understand molecular mechanisms of pathology and to choose the approach for their diagnostics and treatment.
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P-734

Methods of diagnostics and compliancies biological and psychological ages

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Objective: licvidators consequence of the Chernobyl incident with hipertonic disease II-III st. 35 persons – the 1st group, with peptic ulcer of the belly and duodenum 6 persons – the second group, with hypertonic disease II-III st. and peptic ulcer of the belly and duodenum-18 persons – the third group.
Methods: express-method of the determination of the biological age and pictures of health person by change factors electrocinetic potential imcleas of cheekis epithelial hutches, the test "Psychological age of the person".
Results: beside all observed biological age younger chronological, on the most difference in 1^{st} group -11 years; in all group of the observation is noted correspondence to psychological and passport age. In second group psychological age exceeds passport. By comparing relative factors mainly this group form the licvidators consequence of the Chernobyl incident-67.8 %. "Sound" category, where biological age corresponds to passport, in comparison with - licvidators consequence of the Chernobyl incident-25.4%. In greater degree of the detour from rates is revealled in group liquidator, mainly "mentally senior" - 28.8%.
Conclusions: called on study shows that biological age - licvidators consequence of the Chernobyl incident in greater degree is deviate from age rate aside reductions, but psychological age - increasing that is conditioned presence plural psychosomatic pathology, stressful influence of the catastrophe, action ionizing radiations.

P-735

Study of the cells properties using optical tweezers and dielectrophoresis

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In this paper a method for studying optical and dielectrical cells properties using equilibrium between dielectrophoretic and optical force acting on the cell will be presented and discussed. We use red blood cells, a dielectrophoretic chamber with planar electrodes and a laser diode, operating at 830 nm, based optical tweezers. The analytical method for determination of dielectrophoretic force is based on Green s theorem; numerical calculations are made with the software program Mathematica 5.0. A red blood cell is trapped in the optical tweezers at the maximum laser power. After that an electrical field is applied between the electrodes and thus a dielectrophoretic force is exerted on the particle. The power of the laser is decreased very slowly until the cell is pulled out from the trap. At that moment the optical force is equal to dielectrophoretic force which can be theoretically calculated. This procedure is repeated for different amplitudes of the electric field , and the optical force is graphically represented as a function of laser power. The technique of force equilibrium allows the investigation of dielectrical and optical parameters at single cell level such as Claussius Mossotti factor as well as modifications of one of these parameters in pathology or under the action of external agents.

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P-736

He-Ne light transmission through a suspension of red blood cells

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The total attenuation of light by a dilute suspension red blood cells (RBCs) has traditionally been used to study RBCs extinction cross-section. The measured transmitted light intensity is a mixture containing unscattered and scattered photons. Since scattering introduces a systematic error in the measured values, we have make use of several detecting devices: a He-Ne laser coupled with a high resolution CCD camera or a photodiode, respectively and a UV/VIS spectrophotometer, trying to avoid the scattering component.
The collimated transmission is measured in the far field domain with the CCD camera and the photodiode by capturing the light in a small range of angles centered on the laser beam. If the measurements are performed at several meters away from the sample, the scattered component decrease and its relative contribution is drastically diminished. The intensity of the collimated light is determined by fitting the measured light distributions with a Gaussian function for plus a constant offset CCD camera, or is given by the mean photocurrent if the light is captured by the photodiode.
The best results are obtained using the CCD camera. The photocurrent detection is affected by the small far field scattered light background while for UV/VIS measurements the scattering contribution can attain almost 20% in the haematocrit ranges of maximum scattering efficiency.

P-737

The Human transcription/DNA repair factor TFIIH: from Structure to Clinics

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Transcription factor IIH (TFIIH) is one of the best studied basal transcription complexes and is involved in the transcription of practically all mRNA-coding genes as well as in DNA repair. TFIIH is a multi-protein complex composed of 10 subunits, three of which posses enzymatic activities: cdk7, a cyclin-dependant kinase and the two helicases XPB and XPD whose function is to unwind DNA at the transcription start site in the context of transcription initiation or in the vicinity of the lesion in the first steps of the nucleotide excision repair reaction. In human, mutations in the two helicases as well as in the newly discovered p8/TTD-A subunit are directly incriminated in genetically inherited diseases such as Xeroderma Pigmentosum, Cockayne Syndrome or trichothiodystrophy. We have obtained the 3D envelope of Human TFIIH [1] by electron microscopy and have determined either by X-ray crystallography of RMN the atomic structures of isolated modules or subunits [2, 3]. These data provide a structural framework to analyse the function of TFIIH in transcription and/or DNA repair and to discuss the consequences of mutations observed in patients.
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P-738

Potential inhibitors of Staphylococccus aureus bi-components gamma-hemolysins

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S. aureus is one of the major pathogens isolated in hospitals and is responsible for numerous infections. Such infections are particularly dangerous when bacterial strains resistant to antibiotics are isolated. One possible approach that might overcome multi-resistance and avoid the selection of new resistant strains is to diminish virulence of bacteria by blocking their secreted toxins. Common virulence factors of the pathogenic staphylococcal strains are in fact a group of secreted toxins (Hlg, Luk) which attacks host defence cells, thus reducing the immune response of the host. We studied the structure and charge correspondences between toxins and various molecules well tolerated by humans, selecting a panel of about 30 molecules which potentially could interfere with the pore function. We analyzed their ability to block the gamma-hemolysins activity on red blood cells (RBC, both human and rabbit), cultured cells (RAJI) and planar lipid membranes (PLM). The most promising class of molecules is calixarenes as resulted from all the techniques employed in this study. In particular, about 6 μM of 4-sulfonic calix(6)arene can reduce of 50% the hemolytic activity of 12 nM HlgA/HlgB on HRBC. By using PLM experiments we found that calixarenes prevent the opening of new pores possibly by blocking the oligomerization of the toxins, while the block of the preformed pore seems to be to reject. *Sponsored by PAT, project "Stawars".*

P-739

Rod-like cells orientation in alternating electric field – a theoretical study

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The orientation of rod-like cells in alternating electric field was theoretically studied. The interfacial polarization induced by a homogenous alternating electric field determines the orientation of elongated cells either parallel or perpendicular to the field lines in function of the field frequency. The theoretical model presented in this paper takes into account the geometrical and electrical parameters of the cell (size, membrane and cytosol conductivity and permittivity). The torque rotating the polarized cell was calculated and the reorientation frequency vs. electric conductivity of the external medium (the electro-orientation spectrum) was derived. Focusing the model on photoreceptor outer segment behavior in alternating electric field, the disc stack electric properties were considered. The orientation spectrum seems to be very sensitive to membrane permittivity and cytosol conductivity. The last parameter limits the range of external conductivity in which the reorientation can be observed. Also, the spectrum is drastically changed by the width of interdiscal space. Enhancing the membrane permeability (feature of damaged cells) induces a decrease of the internal conductivity. This determines a sharp change in the orientation behavior of cells in low conductive media. We consider that these results can be used in future studies for developing non-invasive methods to analyze the rod-like cells viability.

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P-740

Interaction between model membranes and the anti apoptotic domain BH4

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Cell regulation via programmed cell death is an essential part of life, enabling the body to control its cell population. If this tight regulation fails, pathological cells can escape their fate thereby causing lethal diseases. The origin of these diseases seems to be a serious distortion of the interplay between pro- and anti-apoptotic factors. Pro-apoptotic proteins such as Bax have a similar overall structure except for the BH4 domain.
We therefore synthesized the BH4 domain of the Bcl-2 protein [Khemtemourian, Sani et al., J. Pep. Science, (2005) in press] to investigate the nature of its interactions with membrane models. Using circular dichroism, the peptide is shown to adopt a helical structure in the TFE and is disordered in water. Addition of vesicles (MLVs) leads to a transitional helix conformation that finally results in a β-sheet conformation. Interestingly, when BH4 is pre-incorporated in membranes, it already adopts a β-sheet structure. Using acyl chain perdeuterated DMPC, ^{2}H NMR revealed cholesterol-like action of the pre-incorporated peptide on MLVs. External addition of BH4 seems to adopt an intermediate state between free and membrane-inserted state.
This effect could give a new insight of the anti-apoptotic role of the protein Bcl-2.

P-741

Pathogenesis of COPD (Chronic Obstructive Pulmonary Disease) with Hemorheology, Lipid Peroxidation

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Chronic obstructive pulmonary disease (COPD) is accompanied with both airway and systemic inflammation and oxidative stress. The purpose of this study was to assess the serum concentrations of trace elements that act as a component of oxidative stress in COPD patients. 10 ml blood and 4 ml EDTA added blood were drawn from patiens. Serum concentrations of iron (Fe) ,copper (Cu) and zinc (Zn) were determined using atomic absorption spectrophotometer (AAS-680 SHIMADZU). Relative viscosity was measured in Harkness Capillar Viscometer. Plasma viscosity values determined relative to the viscosity of pure water. The lipid peroxidation product malondialdehyde (MDA) and superoxid dismutase (SOD) in serum samples were measured spectrophotometric methode(UV-VIS-160 A) in terms of TBARS (thiobarbituric acid reactive substances). The results of this study indicate that there are alterations in serum concentrations of trace elements in COPD patients, suggesting that they may play a role in the pathophysiology of this disease by virtue of their role in oxidative stress. **:** As a result of present study it may be concluded that alterations in trace elements and plasma viscosity may be indicator of the COPD.

P-742

Dyslipidemic patients thrombocytes membrane fluidiy

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Platelets are cell fragments in the blood that play an essential role in blood clotting and wound repair; they can also activate certain immune responses. Platelets are formed in the red bone marrow, lungs, and spleen by fragmentation of very large cells known as megakaryocytes. In this work we report a very strong dependency of the blood platelets membrane fluidity (as measured by fluorescence depolarization) on the low density lipid (LDL) content of blood plasma. Correlations are made with other blood parameters associated with different pathologies.
Heparinized blood was collected from both healthy and hyperlipidemic patients. Apyraze (an ADP-ase) was added in order to block platelet aggregation. Samples were diluted 4 times in HBSS (Hank's balanced salt solution) and centrifuged. The pellet was resuspended in HBSS at a cell concentration of 250000 PLT/mL.
An aliquot of 1.5 mL was transferred into a fluorometer cuvette and 5 μL of TMA-DPH 4 mM in DMF was added under agitation and fluorescence anisotropy (r).
A pronounced decrease in fluorescence anisotropy is observed, which corresponds to an increase in membrane fluidity.
Our study reveals that an important physical property of platelet membrane such as membrane fluidity is indeed affected by hyperlipidemia. Subsequent studies are necessary to establish correlations between this parameter, functionality of platelets and consequences on cardiovascular disease and atherothrombotic events.

P-743

Physicochemical characterization and endotoxic activity of synthetic monophosphoryl analogues of lipid A

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It is known that variations in the lipid A moiety of endotoxically highly active lipopolysaccharide (LPS) from enterobacteria may result in dramatic changes in bioactivity. For example, the removal of only one acyl chain in *Escherichia coli*-type hexaacyl lipid A causes a reduction of cytokine induction in human mononuclear cells by 2 to 3 orders of magnitude. In this way, well-directed reduction of the toxic effects of LPS may lead to the development of lipid A-like compounds for clinical use as immunomodulators.
We have performed a physicochemical study on various synthetic diglucosamine phosphate or aminoalkyl glucosamine phosphates with six acyl chains and systematic variations in their lengths and have correlated the data to results in biological test systems. The measurements comprise the determination of the gel to liquid-crystalline phase transition of the acyl chains via FT-IR ans DSC, the aggregate structure of the compounds as found by synchrotron radiation SAXS, their intercalation into target phospholipid liposomes induced by the LPS-binding protein LBP, and their ability to induce tumor necrosis factor-α in human mononuclear cells.The results show characteristic variations of the aggregate structures parallel to the differences in biological activity. In contrast, the acyl chain melting behavior does not correlate directly with the biological data except for a modulating effect.

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P-744

In-vivo proton MRS of brain in children with malnutrition

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In-vivo proton MR spectroscopy at 1.5 T has been used to evaluate the effect of protein energy malnutrition on brain metabolism in children with chronic malnutrition (n=18, age 4-12 years both prior to and after three to six months of nutritional rehabilitation) and age matched healthy controls (n=15). Among malnourished children 13 had normal, 4 had dull normal and one borderline IQ. Interestingly, similar NAA/Cr and Cho/Cr ratios were observed between malnourished and control children in the frontal lobe, cerebellum and basal ganglia. Although, malnourished subjects showed a weight gain of 1.5-4 kgs after nutritional rehabilitation but no change in metabolite ratios was observed compared to controls. Malnutrition is associated with reduction in the number of neurons and demyelination but our results reveal that malnutrition does not have major effect on brain metabolites and hence reinforces the concept of brain sparing at the molecular level in the late onset of chronic malnutrition.

P-745

Crystal structure of Fab fragment of an anti-factor IX antibody 10C12

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Regulated binding of blood coagulation factors onto membrane surface plays important roles in all stages of blood coagulation cascade, including initiation, amplification, termination and regulation. Vitamin K-dependent blood coagulation factor, including factors VII, IX, X, prothrombin, factors C and S, contains a carboxyglutamic acid-rich (Gla) domain at its N-terminal region. In the presence of calcium ion, Gla domain is folded with calcium ions packed at its core and a hydrophobic patch exposed to solvent. Gla domain is thought to be the major anchoring point for blood coagulation factor to bind to membrane surface. 10C12 is a conformation-specific, calcium dependent anti-Factor IX antibody, which is directed at the calcium-stabilized Gla domain and interferes with factor IX-membrane interaction. 10C12 was found to strongly inhibit tenase function and factor IX's binding on endothelial cells and has been demonstrated as an effective anti-coagulant in attenuating thrombosis in several different animal models. We report here the crystallization and structure of the Fab fragment of 10C12. The 10C12 Fab crystallizes in tetragonal space group with unit cell parameters a=159.609Å, b=159.609Å, c=65.366Å. The crystal diffracted to resolution of 3.5Å, and provided conformation of its CDR region.

P-746

Electroporation enhances radiation and drug induced toxicity in murine cancer cells

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Electroporation is a biophysical technique that involves transient increase in the cell membrane permeability due to the application of high voltage electric pulses of short duration. The major challenges in cancer treatment involve the development of radio-resistance and chemoresistance in cancer cells and strong side-effects of the anti-cancer drugs. To overcome these problems, a search for achieving improved treatment protocols is necessitated. In the recent years, cell membrane has been considered as the common target for radiation and electroporation. The present study investigated the effects of combined treatment of the radiation and /or drugs with electroporation on cancer cells both *in vitro* and *in vivo*. The square wave electric pulse generator used in this study was designed, developed and fabricated in the authors' laboratory in BARC. *In vitro* studies have revealed significantly enhanced cytotoxicity when Ehrlich Ascites Carcinoma (EAC) cells were treated with ã-irradiation in combination with square wave electric pulses. Studies also revealed the enhanced apoptosis in EAC cells when drug was applied in combination with electric pulses. *In vivo* studies were performed using the murine fibrosarcoma as the model system. Measurement of tumor growth indicated significant growth delay in case of tumors treated with radiation and /or drug together with electric pulses. The results are promising and suggestive of possible applications in clinics.

P-747

Synthesis and labeling of ferrite magnetic nanoparticles for novel diagnostic applications

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It is becoming increasingly evident that magnetic nanoparticles are a powerful and versatile tool in medicine and biology. More recently, ultrasensitive and rapid magnetic immunoassay techniques are being developed in which the fine magnetic particles were used as labels for antibodies. Antibody tagged magnetic particles were reacted with their antigen, this bound antibody-antigen complex adsorbed on the solid phase, aligned with magnetic field and remanent signal is measured by sensitive magnetometer.
The magnetic nanoparticles ($Mn_{0,5}Zn_{0,5}Fe_2O_4$ ferrite) were synthesized by citrate precursor method. X-ray diffraction pattern of fine particles showed the formation of cubical spinel structure. The SEM micrographs of magnetic nanophase materials were recorded on Jeol 840A SEM, showed spherical, irregular shape particles of size in 20-100 nm range. The ferrite particles obtained by citrate method is small and provide greater flexibility with grain size and size dependent properties. The magnetic particles were coated with bovine serum albumen (BSA) in phosphate buffered saline and with PEG. Monoclonal antibodies against PPR virus inflicting animals are to be coupled with activated magnetic nanobeads. MAB labeled beads will be used for sensitive and rapid detection of respective antigen/ microorganism/ biomolecules.

P-748

Development of DNA Biosensor for detection of 3-nitrobenzanthrone (3-NBA) and its metabolites by SPR and QCM technology

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The 3-nitrobenzanthrone is a very strong bacterial mutagen, present in airborne particulate matter and diesel exhaust, which is well known to induce tumours in animals and is suspected of being mutagenic and carcinogenic in humans. Further, human NADPH: P450 reductase and several isoforms of cytochrome P450, including 2B6, 2D6, 1A1 and 1A2, contribute to the metabolic activation of 3-NBA. Besides this, the cell lines that express different human metabolising enzymes have been tested and showed high genotoxicity and carcinogenicity of 3-NBA and its metabolites.
On the above reported evidences, we have synthesized 3-NBA chemically in our laboratory by its parent compound benzanthrone (BA) and its purity checked by HPLC (80-90%) and to be characterized by UV-VIS, FTIR, Mass, and NMR for target pollutant/carcinogen and then immobilize ssDNA and dsDNA separately on bare gold disk for SPR (Echo Chemie) and Au-Cr crystal for QCM (RQCM, Maxtek, Inc) as probe to detect DNA damage in the form of SPR signal for real time quantitation of DNA as well as mass transfer of DNA in the form of impendence measurement are in progress. Finally fabricate to develop DNA biosensor to detect the toxicity of 3-NBA by recently accepted SPR and QCM technology in biomedical sciences.

P-749

Interaction of pyridinium bis-retinoid (A2E) with bilayer lipid membranes

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Destruction of the rethinal pigment epithelial cells due to accumulation of pyridinium bis-retinoid (A2E) is a cause of age-related macular degeneration. To test its membrane-toxic effect, the interaction of A2E with bilayer lipid membranes (BLM) was studied. Incorporation of charged A2E molecules into membranes was detected as a change of either ζ-potential of multilayer liposomes or boundary potential of BLM. It was shown that presence of up to 25 mole % of A2E in the bilayers from saturated PC did not decrease their stability. The illumination of BLM from unsaturated PC with A2E caused its damage, probably due to oxidation of these lipids by singlet oxygen generated by excited molecules of the pigment. However, this effect was very weak compared to the effect of known photosensitizers. It was found from measurements of boundary potential of BLM that exposure of A2E to light leads to its transforming into at least two products. One of them is epoxy-A2E, which, being hydrophilic, moves from the membrane into water solution, another one is a non- identified hydrophobic substance. The self degradation of A2E under illumination can explain its weak phototoxic effect.

P-750

Crystal Structure of a dimerized plant defensin at atomic resolution

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Plant defensins play an essential role in the first-line defense system of plant against microbial attack. SPE10 is a novel antifungal plant defensin isolated from *Pachyrrhizus erosu,* which exhibit inhibitory activity against several crop-pathogenic fungi. The crystal structure of SPE10, containing 771 non-hydrogen protein atoms and 242 water molecules in an asymmetric unit, has been determined using direct method at an atomic resolution of 0.98Å. The ultrahigh resolution of the experimental diffraction data permits analysis of the structure at atomic level and for the first time a description of water molecules in detail. Comparison of SPE10 with several other defensins or defensin-like peptides revealed some unique characters of SPE10, providing deeper insight into the possible sites involved in specific receptor binding. As the first crystal structure, the packing of SPE10 in crystal also indicates a possible mode for interaction of plant defensin toward membrane.

P-751

Comparison of cachaça and rum response to ionizing radiation

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The irradiation process is presented as an alternative technique in food preservation. “Cachaça”, a sugar cane spirit is a genuine Brazilian product from sugar cane. This research presented the comparison of gamma radiation effects on Brazilian spirit and rum (a similar distillated) irradiated with 0; 150, 300Gy, by means of gas chromatography, pH, Brix and color measurements. The results showed that there was an increase in the acetaldehyde, esters, higher alcohols concentration and Brix scale and a decrease in pH in the irradiated samples reflecting a quality improvement. Also, the content of acetaldehyde, esters, higher alcohols, Brix as well as pH presented high correlation with the radiation dose for both cachaça and rum.

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P-752

Clinical proteomics : characterization and structural studies of new proteins from human body fluids and tissues

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Clinical applications of proteomics involve the use of proteomic technologies at the bedside. By comparing the proteins present in diseased samples with those present in normal samples, it is possible to identify changes in expression of proteins that may potentially be related to organ toxicity. Several structures of a new family of regulatory proteins secreted from the mammary glands during involution that are christined as signalling proteins (SPX-40) from six species have been determined. The structures of these proteins revealed a topology with β/α domain having the triose-phosphate isomerase (TIM) barrel in the core and a small $\alpha + \beta$ domain. These structures are similar to the structures of chitinase and chitinase like proteins. However, these new proteins are unable to bind the carbohydrates but display a unique surface that was found to be involved in protein-protein interactions. A number of peptides with complementary structures to this novel surface binding site of SPX-40 proteins were synthesized and were found binding to these proteins with affinity constants ranging upto 10^{-8} M. The structures of the complexes formed between SPX-40 proteins and designed peptides have also been determined. These structures revealed the positions of peptides close to Trp 191, Asp 186 and Trp 78 and are held through several hydrogen bonds and hydrophobic interactions. These are new findings and have far reaching implications.

P-753

Effect of 50 Hz magnetic fields on yeasts Saccharomyces cerevisae

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A 50 Hz magnetic field effect on the growth of yeasts *Saccharomyces cerevisae* was studied. The cylindrical coil induced magnetic fields with inductions B up to 10mT. Duration of exposure varied up to 24min. Exposure took place at laboratory temperature (20-23°C) and the air ventilator maintained the temperature at the place of sample. We measured growth curves of yeasts in broth by spectrophotometer and the number of CFU (colony forming units) on solid soil. We found that magnetic field decreases the number of yeasts in broth, and slowed their growth down. The effect was stronger for stronger magnetic field inductions and for longer duration of exposure. On the other hand, only small decrease in comparison with control appeared during exposure on solid soil. It seems that the effect depends on the biological age of yeast culture. We observed bigger magnetic field effect after exposure in early stage of evolution of yeasts.
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P-754

ErbB2 signalling in trastuzumab resistant and sensitive cell lines: effect of molecular environment

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ErbB2 is frequently overexpressed in breast and other cancers. Trastuzumab (TR), a humanized antibody against ErbB2 was introduced into clinical practice but was found to have beneficial effect only in $\sim$40% of ErbB2 positive breast tumors. Our hypothesis is that expression levels of ErbB kinases, their interactions and activity within multimolecular complexes and their lipid environment could determine the outcome of ErbB2 directed therapy. Flow cytometric FRET and CLSM measurements on TR resistant (JIMT-1, MKN-7) and sensitive (SKBR-3, N-87) cell lines revealed colocalization and molecular proximity between beta1-integrins and ErbB2, as well as their association with lipid rafts. A weak functional interaction between ErbB2 and β1-integrin (BI) and the fact that ErbB2 did not co-patch with BI upon crosslinking imply that ErbB2 and BI define two functionally distinct molecular association. Although TR-sensitive cell lines expressed more ErbB2 and fewer BI on their surface than their resistant counterparts, this finding probably does not explain the TR resistant phenotype due to the weak interaction between BI and ErbB2. It is proposed that in the resistant cell line active ErbB2 homodimers that bind TR with high affinity are scarce, and proliferation may may be driven by other ErbB kinase dimers such as the ErbB2-ErbB3 heterodimer. Our results imply that the true significance of the expression profile of proteins involved in oncogenesis can only be understood after characterizing their molecular interactions.

P-756

Study of cellular photodamage by flow cytometer after photodynamic therapy on tumor cell line G361

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Photodynamic reactions play important role in processes proceeded in live organisms. It can also be used as suitable treatment tool of cancer diseases.
The purpose of this study was to assess whether PDT induces apoptosis in tumour cell line G361 by using $ZnTPPS_4$ sensitizer and construction of an alternative source of radiation with luminescent diodes (LED). Quantitative analysis was performed by flow cytometer. In our study we used as a fluorescence probe propidium iodide (PI) and Annexin V-FITC Apoptosis Detection Kit (AVADK). Control dishes with G361 cells were exposed to 10 μM camptothecin for 12 hours. Other dishes with $ZnTPPS_4$ were irradiated by LED light source. The cells were treated with the PI and AVADK and analyzed by flow cytometry.
The optimum radiation dose was determinated at 15 J.cm^{-2}. Early apoptosis was evaluated by AVADK and observed 15 - 17 hrs after PDT. 87 % of dead cells were observed 24 hours after PDT treatment.
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LED diod emitter like alternative source of radiation for photodynamic therapy

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Purpose of the work was construction of an alternative source of radation, which will suite with its parametres for initiation of photodynamic phenomenon on carcinoma cells in vitro. In present time is in photodynamic therapy most often used lasers, appriopriately high output lamps with filters. Present apparatus is plant by luminescent diodes emited radiation (LED) in excited wavelengths 455 – 475 nm. Emitter is posible to use with aplication of sensitizer with absorbing maximum in Soret's strip (e.g. for $ZnTPPS_4$). By using of sensitiser with other absorbing spectrum is necessary to use diodes with other emission characteristics. Purpose of these study was find out, whether is possible to induce apoptosis (by using sensitizer $ZnTPPS_4$ and cyclodextrine carrier HP – β – CD) after irradiation of LED emitter on cancer cell line G361. It was proving, that by us constructed source of radiation induce demanded photodynamic response in carcinoma cells. Optimum radiation dose was fixed on 15 $J.cm^{-2}$.
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P-757

Caffeine may alleviate biological activity of MPTP by formation of stacking complexes

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MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin whose effects of action resemble very closely the clinical symptoms of patients suffering from Parkinson's disease. It was demonstrated recently that caffeine has a protective effect on risk of Parkinson's disease in various human populations and attenuates MPTP-induced neurological effects in animal models. Since the effects of caffeine on MPTP-treated animals were mimicked by several antagonists of the adenosine A(2A) receptor, it was suggested that caffeine attenuates MPTP toxicity by blocking this receptor. Here, we demonstrate that caffeine can form stacking (π-π) complexes with MPTP. We found that a biological activity of MPTP (induction of mutations in a microbiological mutagenicity assay), which is completely independent on the A(2A) receptor blockade, is alleviated by caffeine. Therefore, we suggest that reduction of concentration of active MPTP molecules, due to formation of mixed complexes with caffeine, may be an additional mechanism for caffeine-mediated attenuation of MPTP neurotoxicity. Such a mechanism may also operate during protection against Parkinson's disease due to intake of caffeine with diet.

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Intracellular calcium stores contribute to long-term potentiation in sympathetic ganglion of the rat

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Stimulation of presynaptic axons of superior cervical ganglion of the rat with a conditioning train at high frequency produces a long-lasting increase of ganglionic transmission, which is a peripheral form of long-term potentiation (LTP). Many of the cellular mechanisms underlying this LTP remain unknown. We studied the contribution of intracellular calcium stores to the LTP, either by blocking the ryanodine receptor with dantrolene or by evoking the release of calcium with caffeine. We analyzed the effects of these drugs on the amplitude of the postsynaptic voltage responses and on the magnitude and extent of the potentiation. After a conditioning train (40 Hz, 3 s) the maximum amplitude of the postsynaptic response (maximum potentiation) was 3.3±0.2. This potentiated response decreased to 20% of its initial value in 79±13 min (LTP decay time), while the LTP extent (integral of the potentiated response) was 39±5. Both drugs reduced the LTP decay time and the LTP extent in a dose-dependent manner, showing no effect on the maximum potentiation. Thus, caffeine (0.5-10 mM) reduced these parameters between 50-84% and 60-84% of their control values, respectively; while dantrolene (10-100 μM) reduced them 50-70% of control values. Our data demonstrate that intracellular calcium stores contribute to ganglionic LTP; probably by a calcium-induced calcium-release process. (DGAPA IN217702)

P-759

Calculation of the neutron kerma in normal and tumour human tissues

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Neutron kerma of normal and tumour tissues has been calculated using the elemental concentrations. A program developed in Mathcad contains the kerma factors of C, H, O, N, Na, Mg, P, S, Cl, K, etc. that are in normal and tumour human tissues. The program was tested using the elemental composition of tumour tissues such as sarcoma, melanoma, carcinoma and adenoid cystic, also neutron kerma for adipose and muscle tissue for normal adult was calculated. The results are in agreement with those published in literature. The neutron kerma for water was also calculated because in some dosimetric calculations water is used to describe normal and tumor tissues. From this comparison was found that at larger energies kerma factors are approximately the same, but energies less than 100 eV the differences are large.

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HIV fusion inhibitor peptide T-1249 is able to insert or adsorb to lipidic bilayers. Putative correlation with improved efficiency

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T-1249 is a HIV fusion inhibitor peptide under clinical trials. Its interaction with biological membranes models (large unilamellar vesicles) was studied using fluorescence spectroscopy. A gp41 peptide that includes one of the hydrophobic terminals of T-1249, was also studied. Both peptides partition extensively to liquid-crystalline POPC (ΔG = - 7.0 kcal/mol and - 8.7 kcal/mol, for T-1249 and terminal peptide, respectively) and located at the interface of the membrane. T-1249 is essentially in a random coil conformation in this lipidic medium, although a small α-helix contribution is present. When other lipid compositions are used (DPPC, POPG+POPC, and POPC+cholesterol) partition decreases, the most severe effect being the presence of cholesterol. Partition experiments and fluorescence resonance energy transfer analysis show that T-1249 adsorbs to cholesterol-rich membranes. The improved clinical efficiency of T-1249 relative to enfuvirtide (T20) may be related to its bigger partition coefficient and ability to adsorb to rigid lipidic areas on the cell surface, where most receptors are inserted. Moreover, adsorption to the sterol-rich viral membrane helps to increase the local concentration of the inhibitor peptide at the fusion site.

P-761

Katp channel activity is modified by sulfonylurea receptor mutations associated with phhi

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Genetic defects in the K_{atp} channels are associated with a neonatal metabolic disease known as persistent hyperinsulinemic hypoglycaemia symdrome in infancy (PHHI). We characterized the functional consequences of two novel dominant mutations in SUR1 identified in PHHI patients. SUR1 constructs containing point mutations D310N and ΔS1387 subcloned in pECE vector were cotransfected with wild type K_{ir}6.2 in COSm6 cells. Single channel inside-out patch clamp recordings were performed two days after transfection. Both SUR1-ΔS1387 and SUR1-D310N plus K_{ir}6.2 produced functional channels, and mutant channels display an increased activity in response to diazoxide. Deletion ΔS1387 disrupts ADP-mediated modulation of K_{atp} channel activity, but does not alter the inhibition with ATP-Mg. On the other hand, ATP-Mg sensitivity was apparently decreased in K_{ir}6.2/SUR1-D310N channels, but this shift was not observed in the absence of Mg neither in the presence of an analogous not hidrolizable of ATP (AMP-PNP); these mutant channels respond to activation by ADP. This study provides a basis to elucidate the contribution of critical residues of SUR1 in modulation of pancreatic K_{atp} channels by nucleotides, and the molecular mechanisms associated to PHHI

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FCS in cells using different models, applied to interactions of HIV-1 integrase and LEDGF

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HIV-1 integrase (IN) is an essential enzyme for viral replication. Recently we described the interaction of IN with a cellular protein, LEDGF/p75 [1]. We found that LEDGF/p75 enhances the binding of IN to DNA in *in vitro* experiments [2]. In addition we have shown that LEDGF/p75 is required for the nuclear accumulation of IN. We studied the diffusion behaviour of the 3 independent domains of IN and LEDGF/p75 [3]. We found that, for the case of the IN/N-terminal domain, fitting to one-component produced satisfactory results. The data of the other proteins studied had to be fitted by a fast and slow component. Measuring the diffusion of EGFP-IN/core in the presence of over-expressed LEDGF/p75 causes a shift in D showing the interaction between the two proteins.
In order to find out if the slow component was an effect of anomalous diffusion, we fitted the data for normal and anomalous diffusion. In this study we compared both and consider which one is reflecting the real situation in a cell.
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P-763

Study of titin behavior in hibernation: new approach to estimating a stage of human cardiac diseases

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By the use of SDS-electrophoresis in agarose-strengthened 2-2,3% polyacrylamide gels the increase by 12-15% in the content of N2BA titin isoform relative to that of N2B titin isoform in left ventricle (LV) and atrium of hibernating ground squirrels (GS) has been revealed. The expression of titin isoforms with different extensible regions is known to represent the mechanism of regulation of myocardium stiffness. The increase of portion of N2BA titin isoform (with longer extensible region) in myocardium of hibernating GS will lead to the decrease of its stiffness. Such an adaptation must make for the increase of myocardium extensibility and the decrease of heart rate that correlates with the available data. The increase by $\sim$12% of N2BA extent in LV of human heart was also revealed at the first stages of dilated cardiomyopathy (DCM) (Makarenko et al., 2004). The data received from hibernation model are evidence of adaptive nature of titin changes in DCM. Thus the data on titin isoform behavior upon hibernation can be used for estimating the stages of development of DCM and other cardiac diseases and will favor the choice of correct approach to their treatment.
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Picosecond pulse induced two-photon fluorescence enhancement in biological material

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The excitation of two-photon fluorescence (TPF) is a promising detection scheme for biotechnological sensing applications and biophysical studies. We studied the enhancement of two photon fluorescence excitation in fluorescent dyes and fluorescently labeled biomolecules based on resonant grating waveguide structures (DGWS). Picosecond laser pulses generate a large evanescent field based on the guided mode phenomenon in the resonant DGWS, which induces strong TPF signals of the fluorescent dyes Rhodamin B and Lucifer Yellow (LY) at the waveguide surface. Furthermore, enhanced TPF of a LY-labeled human self peptide naturally displayed by major histocompatibility complex molecules of the immune system is demonstrated. An experimental setup was developed which allows to measure membrane proteins and their interaction with fluorescently labeled ligands directly on the DGWS surface in an aqueous environment, thereby strongly encouraging the use of DGWS as enhancement platforms in spectroscopic applications for membrane proteins, biological membranes and cells. Applications to investigate differential autoimmune disease association by two antigen presenting molecules will be presented [1].
[1] Pöhlmann, T., et al. *J. Biol. Chem.* **279**, (2004) 28197

P-765

Elucidation of antigen dissociation pathways: Entropic control of peptide recognition?

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Peptide presentation by major histocompatibility complex (MHC) molecules is of central importance for immune responses, which are triggered through recognition of peptide-loaded MHC molecules (pMHC) by cellular ligands such as T-cell receptors (TCR). In this study two pMHC molecules are compared which exhibit differential autoimmune disease association (ankylosing spondylitis) and differ only in one amino acid (B*2705: disease-associated, B*2709: nonassociated). When loaded with the same peptide, all structural, dynamic, and functional differences between these subtypes are due to the exchange of the single amino acid in the peptide binding groove which is not accessible to a TCR. Recently, differential peptide dynamics were found despite virtually identical crystal structures, suggesting an entropic control of peptide recognition (1). Here, we have focused on the kinetics and thermodynamics of peptide dissociation and MHC complex unfolding of the two subtypes using fluorescently labeled nonapeptides (m9) and steady-state and time-resolved fluorescence depolarization. Despite subtype specific differences, a common pathway for peptide dissociation was elucidated.
(1) Pöhlmann, T., Böckmann, R.A., Grubmüller, H., Uchanska-Ziegler, B., Ziegler, A., and Alexiev, U. *JBC* **279**, (2004) 28197

P-766

Defective neuron-neuronal synapses in mice lacking amyloid precursor protein and APP-like protein 2

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Although abnormal processing of amyloid precursor protein (APP) leads to early onset of Alzheimer's disease, the normal function of this protein remains unclear. Mice homozygous for APP or its homologue APP-like protein 2 (APLP2) null mutation (KO) are viable, but double mutants for APP and APLP2 deletions (DKO) are early postnatal lethal. APP is widely expressed in both central and peripheral nervous systems. To investigate the role of APP in synapse development, we compared the ultrastructure of submandibular ganglion synapses between DKO and littermate APLP2 KO mice at birth using serial electron microscopy. We found that the size of presynaptic boutons and the number of active zones were comparable in both strains of animals. However, the synaptic vesicle density, active zone size, and the number of docked vesicles per active zone were significantly reduced in DKO compared to those in APLP2 KO. Together with our previous findings of reduced synaptic vesicle density and defective neurotransmitter release in neuromuscular junctions in DKO, our results suggest that APP family of proteins play an important role in the development of synaptic structure and function throughout the nervous system.

P-768

Variations of signal transduction of cells in animals exposed in electromagnetic field irradiated by high-voltage transmission lines

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The influence of low-frequency electromagnetic field (EMF) of high-voltage transmission lines(HVTL) on JAK-STAT signal transduction of cell in rates are studied. The rats in 4^{th} weeks are divided into controlled and exposed groups. The experimental group is exposed in EMF about 400 days. The EMF is generated by an appliance. The strengths of the electric and magnetic fields are 4000 V/m and 0.09-0.1 G, which are the strengths of the electric and magnetic fields at the position of 2.0-2.3 m distancing the earth under HVTL of 220 KV. The quantity of Phosphorylation of signal transducer and activator of transcription 3 (STAT3) extracted from spleen cells stimulated and unstimulated by IL-2, respectively, are detected by the immunoblotting and immunobiochemistey. The results show that the expression of phospho-STAT3 in cell stimulated by IL-2 in exposed groups increases more significantly ($P < 0.05$). This shows that signal transduction of cell is affected by EMF. Spectra of infrared absorption for proteins participating this signal transduction of cell for the controlled and exposed groups, which are measured by FT-IR spectrometer, are obviously different both the intensity and frequency. This shows that molecular structure of the proteins changes under influence of EMF of HVTL, which results just to above changes of the JAK-STAT signal transduction of cell.

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Variations of signal transduction of cells in animals exposed in electromagnetic field irradiated by high-voltage transmission lines

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P-769

Influence of the membrane fluidity in the interaction with hepatisis G synthetic peptides

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Interaction of hepatitis G virus (HGV/GBV-C) with cells during infection and immunological response is not yet well-known. It seems that hydrophobic and electrostatic interactions with the membrane lipids play an important role. In order to have a better understanding of the fusion mechanism between the virus and membrane cell, we have used biophysical techniques to study the interaction between two synthetic hepatitis G peptides and phospholipids. Two peptides of different lenght (E2(347-363) and E2(354-363)) have been selected to determine the influence of the aminoacid sequence. In the present study we have focussed in determininf the influence of the membrane fluidity in the interaction of theses peptides Therefore, we have selected as lipid to study, dipalmitoyl phosphatidylcholine (DPPC) and a more fluid phospholipid palmitoyloleylphosphatidylcholine (POPC).
Biophysical studies included monolayers (kinetics at constant area) and bilayers (DSC). Previously the surface behaviour of the peptides was determined through their surface activity and ability to forms stable monolayers at the air/water interface.
Both peptides interact with DPPC and POPC, even though the monolayer studies were more effective in detecting the interaction. Fluidity and longuer sequence were found to increase the degree of interaction.

P-770

Chemiluminescence analysis of action of anti-inflammatory preparations on the lipid peroxidation

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Process of free radical oxidation plays an important role in the normal functioning of organisms. Under presence of some factors this process may be disturbed. In contemporary medicine for treatment of different deceases there are used many pharmacological preparations, mechanisms of action of which is not very clear. The aim of this work is to observe the alteration of lipid peroxidation under influence of some anti-inflammatory preparations such as paracetamol, aspirin, analgin, and 5-NOK. For this purpose there are usually used methods, which allow revealing the changes of antioxidant level in the organism.
For our experiments we provided the chemiluminescence analysis with the use of homogenate of brain of cow at the quantometric devise, which worked at the basis of photomultiplier-139 (t=20-40^{0}C). The results marked that anti-inflammatory preparations inhibited the level of chemiluminescence of samples, which reflected the inhibition of free radical oxidation process. Thus, used preparations behaved as antioxidants and traps for free radicals. It was found that those preparations had different levels of such activities. Thus, the highest effect was registered in the presence of 5-NOK (60%), and the smallest – paracetamol (25%).
These results might be useful for understanding the physical-chemical aspects of nature and mechanisms of action of pharmacological preparations while reacting with bio-structures.

P-771

Study on effects of bioelectric parameters of rats in EM radiation of HV transmission line

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To study the effects of bioelectric parameters of rats in the electromagnetic radiations of high-voltage transmission line, EEG, ECG and CMAP were measured in rats exposed to simulating HV transmission line EM radiation for over one year. Brain tissues were studied by Fourier transform infrared spectroscopy. Results showed that no significant difference between exposed group and control group in power spectrum of EEG. However the FT-IR spectra of brain tissues were different. A peak of IR spectra of brain tissues in exposed group was observed at 2345 cm^{-1} but no in control group, which indicated that oxygenic metabolize in rats' brain exposed to EM radiation of simulating HV transmission line might be influenced by EM radiation somehow. ECG of the exposed animals was considerably altered. The heart rate changed from 462$\pm$26 beat/min in control group to 275$\pm$30 beat/min in exposed group; duration of P wave prolonged from 12.1$\pm$1.2ms to 20.2$\pm$2.0ms; duration of T wave Prolonged from 69.8$\pm$4.3ms to 84.8$\pm$3.8ms; amplitude of T wave increased from 0.08$\pm$0.01mV to 0.14$\pm$0.04mV. These changes were significant (P<0.05 or P <0.01). The latent period of CMAP in exposed group were not different compared with control group however wave amplitude of CMAP changed from 6.26$\pm$0.55mV in control group to 4.21$\pm$0.66mV in exposed group significantly. All results indicated that there must be some effects on bioelectric parameters of rats exposed to EM radiation of HV transmission line for a long time.

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Tissue observation of rats in EM radiation of HV transmission line by infrared spectroscopy

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Objective: To study the long-term effects on human body exposed to electromagnetic radiation of high-voltage transmission line.
Methods: Tissue's pathological changes in rats, which were exposed to electromagnetic radiation of simulating high-voltage transmission line for over one year, were observed and analyzed. Synchronously, paraffin sections were studied by Microscopic Fourier Transform Infrared Spectroscopy technique on lung tissue and kidney tissue of exposed group and control group. Results: The peaks of IR spectra of lung tissues in control group were observed at 1659 cm^{-1} and 1548cm^{-1} but not in exposed group, which indicated that proteins in lung tissues exposed to electromagnetic radiation of high-voltage transmission line might change conformations and disorder structures with their molecular hydrogen bonds nearly broken. Some spontaneous lesions were observed in 2 cases of 8 lungs of exposed male rats, including inflammatory cell infiltration, degenerations, abscess, and fibrosis.
Conclusion: The results showed that there were some differences between exposed group and control group on both lung tissue sections and IR spectra, which was mainly due to changes in composition and structure of micro-molecules and changes of vibrational modes of the function groups in biological tissues. It indicated that hydrogen bonds in proteins might be broken.

P-773

Ionic mechanisms of the rate independence for the ischemic cell in heart

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Computer simulation method was used to study ionic mechanisms of the rate independence for the cardiac ischemic cell. A dynamic ionic model of the ventricular action potential was used in the study. The ischemic cell was simulated by decreasing the intracellular ATP concentration, reducing conductance of the inward Na^+ current, and increasing the extracellular K^+ concentration. The forth-order Runge-Kutta method with adaptive time step was used to solve the constructed models. The numerical experiments showed that different from physiological characters of the normal cell, the action potential durations of ischemic cells did not change with beats of shorter or longer cycle length. By comparing some transmembrane currents I_k (time-dependent delayed K^+ current), I_{CaL} (L-type calcium current), and I_{NaCa} (Na^+-Ca^{2+} exchange current) that could play the important role during the plateau and repolarization phases of the action potential, it was found the less increased I_k and less outward I_{NaCa} at the onset of depolarization were the ionic substrates of the less rate dependence for ischemic cells. In addition, it proved that with the depressed Na^+ current, the I_{CaL} was significantly larger in ischemic cells, which led to smaller upstroke velocity of the action potential under the ischemic condition.

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Comparative study of the properties in ischemic fibers with different transition forms

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Simulated regional ischemic models with abrupt transition between normal and ischemic region are often used to study mechanisms of arrhythmia. Whether the simplified models could better approximate the real ischemic myocardium was our concern in this paper. Two kinds of regional ischemic fibers were constructed in our simulation research. One had abrupt change between normal and ischemic region. The other transition was gradual with linear change. The ischemic cells were developed by decreasing the intracellular ATP concentration, reducing conductance of the inward Na^+ current and increasing the extracellular K^+ concentration. The operator splitting method was used to integrate the models. The duration, resting potential, and amplitude of the cellular action potential along gradually changed fiber was in between the corresponding value for normal and severely ischemic cell in sudden transitional fiber. The conduction velocity of the wave slowed while the width of the vulnerable window increased with the ischemic aggravation. It proved that due to electrotonic interaction the transitional portion actually existed even if the transition form was abrupt. The two models had similar properties. The models with abrupt transition could not influence the qualitative results of the simulation study.

P-775

The biological effect of nano-iron oxide and its hydrate

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Nano-iron oxide and its hydrate were synthesized by hydrothermal method and confirmed by infrared spectrum and SEM (Scanning Electron Microscope) et al. The dimension of the particulates of iron oxide and its hydrate are about 50-120 nm. The shape of nano-iron oxide is like globosity while its hydrate rod. Amino acids intermingling with the synthesized nanomaterials were crystallized to investigate the space effect of nano-particulates. The shapes of crystal are different to that of pure amino acid. The positions and width of nanomaterical's peaks in the infrared spectrum are changed also. Microscope observation and infrared spectrum results indicated the nanomaterials had changed the internal structure of amino acids crystal. To considerate the toxicity of the synthesized nanomaterials, MTT (3-(4,5-dimethylthiazol 2-yl)-2,5 diphenyltetrazolium bromide) assay was used to determine their cytotoxicity. The OD value (Optical Density) was used to calculated RGR% (Relative Generation Rate) of cells, which determined the grade of cytotoxicity. The RGR of nano-patiulates of iron oxide and its hydrate are about 1 to 2, which indicate they have just low toxicity. Nanomaterials with different dimension will be synthesized and assayed in the future. It will help us to find the proper size of nanobiomaterials for the using of biomedical engineering.

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– Biophysics and Disease –

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Biological toxicity of the carbon nanotubes

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The influences of the carbon nanotubes on proliferation state of chick embryo fibroblast (CEF) cells and toxicity of the nanotubes to the cells have been investigated by 3-(4,5- dimethylthiazol 2-yl)-2,5 diphenyltetrazolium bromide (MTT) colorimetric method and the toxicology. The carbon nanotubes are made by using a thermally chemical vapor deposition method in a gas mixture of nitrogen, hydrogen and acetylene. The diameters of the carbon nanotubes are measured by Scan- ning Electron Microscope and Transmission Electron Microscope, and are 100nm. These cells are divided into controlled and experimental groups. We found experimentally that the values of the cell proliferation rate of the CEF cells in the experimental group are very small, but the values of its relative cell proliferation rate are larger under the action of the carbon nanotubes when compared with that of the controlled group. This shows clearly that the influences of the carbon nanotubes on the proliferations of the CEF cells is very small, it is about first score.

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– Molecular Crowding –

P-780

Membrane-catalyzed nucleotide exchange on DnaA protein: effect of surface molecular crowding

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DnaA is the initiator protein of chromosomal replication in bacteria; it specifically recognizes the origin and recruits the replication machinery to this site. The activity of DnaA plays a central role in the timing of primary initiations within the *E.coli* cell cycle. A controlled, reversible conversion between the active ATP-DnaA and the inactive ADP form modulates this activity. In a DNA-dependent manner, bound ATP is hydrolyzed to ADP. Acidic phospholipids with unsaturated fatty acids are capable of reactivating ADP-DnaA by promoting the release of the tightly bound ADP. We are studying the molecular mechanism of DnaA-membrane interaction and how this interaction modulates the DnaA-nucleotide binding using fluorescence-spectroscopy methods. The nucleotide binding kinetics, measured with the fluorescent derivative MANT-ATP, was highly temperature dependent. MANT-ATP dissociation rate constant depended on DnaA density on the membrane in a sigmoidal manner: it increased three-fold when the protein density was decreased. At high densities the nucleotide was completely released due to protein exchange on the membrane, despite a small fraction of protein interacting with the membrane. These effects are ascribed to local macromolecular crowding on the membrane surface as supported by the influence of temperature and neutral polysaccharides on the reaction.

P-781

Fuzzy nanostructured polyelectrolyte (FNP) fluorescence layers and shells in molecular biophysics

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3D fluorescence microscopy offers a wide spectrum of tools useful for molecular biology like FRET, FRAP, or FLIM to investigate molecular processes in model systems or even in living cells. Model systems often have the drawback to facilitate the problem too much and not taking into account molecular crowding effects. FNP layers or shells made by LbL technique allow to fill the gap between too simple model systems and the complexity of cells. They can be used to investigate molecular interactions in increasing crowded environments by means of immobilization of visible fluorescent proteins and molecules. FNP's permeability is strongly influenced by salt concentration and pH while layer deposition demanding for design optimization as function of the application. Hence, it is an interesting system for diffusion studies and for molecular interactions. Tunability of permeability and of layer thickness make the system particularly well suited for FRAP and FRET studies. Besides the application to biological systems and the investigation of basic properties the polyelectrolyte systems offer a convenient ability to check elementary properties of the used microscope, including the utilization of such a system as testbed for FLIM calibrations. Experiments were performed utilizing confocal laser scanning microscopy, multiphoton microscopy and TIRF.

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MCE and CE results for chemical modifications vs mutational in determining valence

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Capillary electrophoresis (CE) has been used to compare the mobility of charge ladders of T4 lysozyme produced in two ways: by chemical modification (acylation of lysine residues) and by mutation. Valence determinations made solely from assumed differences in net charge (the "charge ladder method") are compared with values calculated from mobility using simple Debye-Hückel theory, the later requiring the use of the hydrodynamic radius. Sedimentation velocity studies were done to compare the hydrodynamic radius of the charge ladder to that of each individual mutant. The radius was also calculated from the charge ladder method for comparison. Furthermore, charge was measured by membrane confined electrophoresis (MCE) under the same conditions and found to be in excellent agreement with CE results. There has been some question as to whether the use of a single radius for all species is valid in valence calculations derived from the charge ladder method as well as whether charge regularization might limit its utility. Results given here suggest that, accurate charge values can be obtained using a single radius and that charge regularization is minimal in the range.

P-783

Calculation of molecular crowding effect between two actin molecules making contact

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One of interactions between macromolecules is the attractive mean force through excluded volume effect, which has been studied recently in colloid, polymer, and virus sciences and is also called as depletion force. We studied the attractive mean force between actin molecules. Actin molecules associate each other and form filaments in natural condition in a cell. The formation of filaments is thought to be the origin of cell movement which is called as "cell crawling". In order to reveal the mechanism of the filament formation, we calculated potential of mean force between two actin molecules through excluded volume effect from free actin monomers by using extended scaled particle theory (XSPT) which has been developed by us. At first, we calculated two suspended actin molecules as two spherical molecules. Calculated differences of the values of the attraction potential of two molecules between at contact and at one molecule apart by using XSPT is almost the same as those by Asakura-Oosawa theory. In the next step, we treated each suspended actin molecule has an arbitrary shape, which is regarded as fused spheres consisting of a sphere of a heavy atom. Increase of the density of free actin monomers stabilizes the actin dimmer. Our results indicate that an actin dimmer favors the Lorenz 2 configuration where two actin molecules are located along the spiral path of the filament. This result supports the experimental results of configurations of an actin dimmer.

Posters

– Molecular Crowding –

P-784

Monte Carlo simulation of enzymatic reactions in 2D and 3D crowded media

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The fractal-like approach for the enzyme kinetics considers that the rate coefficients for the diffusion controlled reactions are not constants but they are depending on time. We apply fractal kinetics approach using a Monte Carlo simulation for studying Michaelis Menten enzymatic kinetics in a 2D and 3D lattices with obstacles following Berry's algorithm. We apply this algorithm for situations more physically realistic in the cellular media, as different degrees of mobility and different sizes for big molecules and obstacles. These simulations suggest that in crowded media we always deal with a fractal-like kinetics, but with different types of fractality.

P-785

Neutron scattering study of nucleic acids dynamics in cellula

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Neutron scattering experiments have been performed on whole natural bacterial cells and established the biological significance of dynamics on a macromolecular level (Tehei and al, 2004). Two parameters were measured: mean square displacement, which corresponds to macromolecular flexibility that is essential to the biological function, and resilience, which corresponds to an effective force constant associated to the structure stability (Zaccai, 2000). Both parameters referred to the average contribution of all macromolecules inside cells and reflected how average dynamics had adapted to the bacterial physiological temperature. Neutron scattering cross-sections are significantly different for H and ^{2}H(D) and selective H-D labelling can be used to examine dynamics in different parts of a cell. In the present work, dynamics of *Escherichia coli* RNA components were investigated *in vivo*, by selective H-labelling of nucleic acids in D-cells. Analytical centrifugation and mass spectrometry were used to control H-isotope level in the RNA and proteins.

P-785-B

Spectroscopics properties of nevaminoglcosyl porphyrins

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Photodynamic therapy cancer-against is based on the principle that porphyrins become concentrated in tumor cells and, upon subsequent irradiation with visible light in the presence of Oxygen, specificially destroys the cell. It appears that porphyrins with sugar moieties have not only good solubility in aqueous solutions but also possible specific membrane interaction.
From this perspective and as a first approach, we have synthesized mono, di and tri alanine glycoporphyrin derivatives; two tristolylporphyrins substitued by one glycosylated serine because the presence of lipophilic aryl groups and hydrophyric substituants could increase the interaction with lipid membranes. After that, we have studied the fluorescence emission and excitation spectra of these products in order to have conlusions for possibilities to induce the oxygen singulet; the dimeric structure of these products can be discussed beginning from the exciton theory.
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P-786

Self- and collective diffusion of selected model components of complex system. Size and shape effect

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Biopolymers constitute ideal model particles for fundamental research because of theirs monodispersity and diversity of shapes and sizes. Independently, the diffusive rates of biomacromolecules in non-dilute suspensions are important factors alone, since many biological processes involve suspensions in which the particles occupy a significant fraction of the total volume and, as a result, they significantly interact with each other. We present here measurements of the long time self- diffusion coefficients and collective diffusion of various biopolymers, different in size and shape, over wide concentration and ionic strength range. Special attention is paid to the low salt solutions and low concentrations where the electrostatic interactions are not screened. The self-diffusion coefficient decreases monotonically with increasing concentration and with decreasing ionic strength. However, at lower added salt concentration one observes non-monotonic decay of the diffusion coefficient for some of the samples (i.e. 20 base pair oligonucleotide). Finally, the FCS self-diffusion coefficients are compared with the mutual diffusion coefficients measured by DLS and the effect of electrostatic and hydrodynamic interaction on both diffusion coefficient as well as the relation between them is discussed.

Posters

– Molecular Crowding –

P-787

Tracer diffusion of proteins in a crowded environment

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The interior of cells in all living organism, without exception, has a common feature: the high concentration of macromolecules they contain, which occupy a considerable fraction (20-30%) of the total volume [Ellis, *TIBS* 2001; Minton , *JBC* 2001]. The diffusion of macromolecules within this crowded environment is an essential requirement for crucial biological processes such as metabolism, transport of solutes, protein processing and signaling events. In this work we have determined the diffusion coefficients of apomyoglobin (apoMb) in solutions containing different concentrations of RNase A and HSA (25-250 mg/mL) as crowders, by using time-resolved fluorescence anisotropy (TRFA) and fluorescence correlation spectroscopy (FCS). The determined diffusion times have been interpreted in terms of local rotational and translational viscosities, molecular parameters characteristic for each tracer/crowder system. Quantitative studies like those presented in this work will contribute to understand how biomolecular diffusion is modified by the presence of other macromolecules, which occupy a substantial fraction of the total volume.

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