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Investigations of polypeptide topology and rotational diffusion in membranes by solid-state NMR

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Here we present a novel solid-state NMR approach which allows for the accurate determination of the tilt and rotational pitch angles of peptides reconstituted into uniaxially oriented membranes. The method works with transmembrane or in-plane oriented peptides that have been labelled with 3,3,3-$^{2}H_3$-alanine and ^{15}N-leucine at two selected sites. Proton-decoupled ^{15}N and ^{2}H solid-state NMR spectroscopy at sample orientations of the membrane normal parallel to the magnetic field direction have been used to characterize the tilt and rotational pitch angle of these peptides in considerable detail.
The same samples when inserted into the magnetic field at 90 degrees tilted alignments provide valuable information on the rotational diffusion constants in membranes and thereby of the association and size of peptide complexes within the membrane environments. Whereas monomeric transmembrane peptides exhibit spectral averaging and well-defined resonances, larger complexes are characterized by broad spectral line shapes. In particular the deuterium line shape is sensitive to association of a few transmembrane helices. In contrast, the formation of much larger complexes affects the ^{15}N chemical shift spectrum.
Aisenbrey Ch. & Bechinger, B. *Biochemistry* 43, 10502-10512 (2004)
Aisenbrey Ch. & Bechinger, B, *JACS* 126, 16676-16683 (2004)
Bechinger, B. *BBA* 1666, 190-204 (2004)

P-789

Translocation of amino acids from the membrane interface to the interior: Theory and Experimiment

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The interactions of a series of histidine-containing peptides with biological model membranes have been investigated by attenuated total reflection Fourier transform infra red (ATR-FTIR) and oriented solid-state NMR spectroscopies. Related peptides have previously been shown to exhibit antibiotic and DNA transfection activities. The 26-residue LAH_4X_4 and LAH_4X_6 peptides were designed in such a manner to form amphipathic helical structures in membrane environments. Four histidines and four/six variable amino acids X constitute one face of the helix whereas leucines and alanines characterize the opposite hydrophobic surface. The dichroic ratio of ATR-FTIR spectra, or the orientation-dependent ^{15}N solid-state NMR chemical shift have been used to follow the pH-dependent transition from in-plane to transmembrane alignments upon increase in pH. A theoretical model of the topological modulations is presented and the experimental transition curves analysed in order to reveal the Gibbs free energy of transition. The novel concept provides access to the free energy changes associated with the amino acids X incorporated into an extended α-helix and in the context of phospholipid bilayers. For the peptides of the LAH_4X_4 series the Gibbs free energies associated with the transition from the membrane interface to the bilayer interior follow the sequence of amino acids: L < A $\approx$ I < S $\approx$ F < T $\approx$ G < V $\approx$ W << Y. (Bechinger et al., *Biophys. J. 76*, 552-563 (1999) and Aisenbrey et al.; submitted)

P-790

Structural and functional studies of the S1 ribosomal protein RNA binding region

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In order to understand mRNA stability, we proceed to the studie of life time model system composed of the RegB ribonuclease and the ribosomal protein S1. The T4 bacteriophage life cycle is modulated by RegB which mediate specific mRNA degradations. RegB cleaved the mRNA at the middle of the translation initiation region (Shine-Dalgarno SD). Studies had shown that RegB activity is enhanced with addition of S1 which is normaly required for the translation of mRNA in case of unusual SD regions. S1 is considered as a key factor of translation's inititiation. Composed of six similar domains (F1-F6), it interacts with the ribosome by his F1-F2 domains and with the mRNA by F3-F6. We had shown that the F345 fragments got the same properties than the whole protein. Many global architectures had been published (linear or globular conformation). Then we try to understand what is the catalysis way of S1 in the RegB activation and in RNA recognition. We study the global conformation of F345 fragment. For that, we use NMR to determinate the domains interfaces. At first we made the NMR backbone assignement of 15N/13C/2H labeled fragments (F34-F45). Then we analysed HSQC overlapping of each fragments in order to identify interfaces residues. The residues identification on S1 homologous model allowed us to determinate interaction region between domains. Currently we study of S1-RNA interactions to determinate if the ligand conformation could change the interaction region and involve conformationals changes.

P-791

A new high pressure SAXS cell for protein denaturation studies on microvolumes

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Protein folding studies are essential to understand the relationship between gene sequence and protein function.
Proteins denature with increasing temperature, chemical denaturant concentration or pressure, among which pressure unfolding is the mildest, most reversible method. The protein's global size and shape changes during unfolding can be monitored by Small Angle X-ray Scattering (SAXS) in solution. As SAXS data acquisition requires high protein concentration hence small sample volume, a new microvolume high pressure cell has been designed for synchrotron SAXS analysis. The sample is enclosed in a grease-plugged 20μl plastic cell with kapton windows, which isolates the sample from the pressure medium while transmitting pressure to the sample without a change in sample pathlength. The inner cell is then placed in water between 2 diamond windows in the pressure cell for pressurization. The pressure range is 0 to 3.5kbar.
As a first test, we studied the behaviour of bovine beta-lactoglobulin (bLG) and horse skeletal muscle holomyoglobin (HMB) under pressure. bLG unfolding was partially reversible under pressure. HMB at pH 9 proved to be sensitive to radiation damage, but radiation effects could be distinguished from pressure effects. HMB denaturation under pressure was mostly irreversible.

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Interactions involving helices from membrane proteins:energetics of assembly and folding

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The structure of soluble proteines has been widely analysed in order to understand the principles of their underlying organisation. Membrane proteines however have not been subjected to extensive analysis in part because barely 400 of the 30000 structures in the pdb are membrane proteins. About 3/4 of these are alpha helical transmembrane proteins, the dominant class of integral membrane proteins. The two step model has been proposed as a thermodynamical model that explains the folding of these proteins in two steps: first, the insertion of transmembrane segments and the formation of helices. Second the assembly of these preformed helices into the helical bundle that constitues the protein. To gain further insight into the second step of this model and probe the interactions between these helices we have examined the energetics of helix interactions. A non-redundant subset of helical membrane proteins has been analysed using an optimized forcefield and the enthalpies of helix-helix interactions extracted and analysed.

P-793

Fractal correlation dimensionality and pattern formation - proteomic and genomic application

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The similarity of patterns identified in mathematical, physical and biological objects suggests that common means might exist for their studies. The geometrical mosaic objects, the physical events of phase separation and the linear heterogeneous biopolymers forming clusters seem to be the examples. A fractal appears to be a good model to be used in such studies. During the fractal construction, a part of the original geometrical space is removed. The removed part comprises the fractal complement. We compare Monte-Carlo simulations of a two-dimensional Sierpinski gasket and one-dimensional Cantor set to see if the fractal correlation dimension might be different for a fractal and the complement fractal. Computations of the fractal correlation dimension of the Cantor set d_F, and that of the fractal complement, d_C, show a finite gap of dimensionality $\Delta d = d_C - d_F \approx 0.3$. In the simulation of the Sierpinski gasket, which is a 2D analogue of the Cantor set, the gap tends to zero with the number of generations increase. We invoke the Landau theorem about impossibility of the equilibrium coexistence of macroscopic physical phases in linear physical systems to propose that the difference fractal correlation dimension may be the geometrical implication of that condition. We are referring to several linear biological objects exhibiting higher dimension mosaic structure, to discuss a possible application of the fractal correlation dimensionality in structural genomics.

P-794

Key role of Asn2 in the formation of native disulfide bonds. x-ray structure of apamin

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The X-ray structure of the N-acetylated bee venom neurotoxin apamin (C1-C11, C3-C15 disulfide bridges) has been determined at 0.9 Å resolution. It mainly consists of a beta-turn (residues 2-5) and of an alpha-helix (residues 9-18). The beta-turn is stabilized both by the classical i, i+3 hydrogen bond and by an additional one involving the N2 side chain and K4 amide proton.
In order to assess the role of N2 in the oxidative folding, it was replaced by the isosteric but hydrophobic Abu residue. In contrast to apamin, that quantitatively yields the native disulfide pattern, the [Abu2]-apamin analogue yielded not only the apamin-type disulfide pattern (60%) but also the endothelin (C1-C15, C3-C11) pattern (40%). NMR structural studies of these two isomers revealed that the A5-P6 amide bond mainly adopts a cis conformation. Our results support the essential role of the N2 hydrogen bond, both in the yield of the native pattern and in the conformation of the A5-P6 amide bond of apamin.

P-795

Topological signatures of good folders

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We study the topology of the energetic landscape of off-lattice protein models in order to determine the structural features that distinguish aminoacidic sequences with good folding propensities. The dynamical connectivity between the basins of attraction of different energy minima is accessed by the identification of the first order saddles dividing them. A statistical analysis of the configurational distance between connected minima shows that the motion on the energy landscape consists in a series of many short activated jumps, long jumps being relatively rare. In this respect the steepness of the energy funnel shows not to be the only factor that drives folding. Though good and moderate folders may show very similar funnels, in sequences characterized by a good folding propensity the probability of long configurational jumps shows to be higher than in other cases. The analysis is complemented by the evaluation of geometric quantities related to the landscape curvature.

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Kinetic bottlenecks identification in different folding models

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The WW domains are a family of fast folding, compact, modular domains featuring a triple-stranded, antiparallel beta-sheet. The WW domain of the Pin1 protein, due to the availability of a complete picture of the residues involved in thermodynamic stability and in the formation of the transition state, in particular, represents an excellent benchmark to test computational methods. The objective of the present work is to identify the kinetic bottlenecks in the folding process through MD unfolding simulations at increasing temperatures. The kinetic bottlenecks are related to the establishment of contacts requiring the overcoming of a large entropy barrier and acting as a nucleus for the creation of further contacts. The key sites are therefore those involved in contacts showing a dramatic decrease in fractional occupation near the specific heat peak. The technique was applied to the Go model and to a model based on the knowledge of secondary structure, providing in both cases a picture of the folding process consistent with the experimental data. Evidence is also shown that while the Go model allows a more accurate prediction of the native structure, the folding pathway is better described by the other model.

P-797

Structure and dynamics of the near native excited states of the dynein light chain protein

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Dynein light chain (DDLC1), a member of the cytoplasmic motor assembly exists as a monomer or a dimer (functional form) under different experimental conditions. Here we report the unfolding characteristics of the monomeric DDLC1 at pH 3, due to urea and guanidine hydrochloride, by various biophysical techniques. It is observed that the unfolding pathways due to the two denaturants have many differences. Urea unfolding seems to be two state, while guanidine unfolding is more complex. The NMR experiments carried out at low denaturant concentrations have enabled detailed characterization of the structure and dynamics of the near native excited states of the protein. These are similar to the native state in structure, except for the small extensions of the helices in the N-terminal half of the protein. However, the local stabilities of the α and β-strands are perturbed and this occurs differently in the two denaturants. In the guanidine case the entire multi-stranded β-sheet in the C-terminal half is destabilized. In either case the motional characteristics, seem to suggest the presence of a finite population of the dimer in the excited state ensemble. These states are suggested to be likely intermediates in the momoner-dimer transition, and their characterization here thus provides clues to the molecular mechanism of the transition. It is also envisaged that the near native excited states could play regulatory roles in the functioning of the protein.

P-798

Structural-based differential stability in the YoeB-YefM toxin-antitoxin module

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The specific physiological role of natively unfolded proteins is only recently beginning to be explored. A notable case in which natively unfolded state appears to have physiological significance is the *E. coli* YoeB-YefM toxin-antitoxin (TA) module. A crucial element in proper functioning of TA systems requires physiological instability of the antitoxin in contrast to the stable profile of its toxin partner. We have shown that YefM antitoxin is a natively unfolded protein, lacking secondary structure even at low temperatures. In contrast, its toxin partner has a well-folded conformation at physiological temperatures. We suggest that the structural-based differential thermodynamic stability between the two components is the cause for their differential physiological stability, since structural instability of the antitoxin exposes it to cellular quality-control machinery. We further revealed that YefM and YoeB interact and form tight complex and determined it stoichiometry.
A potential use of TA systems is as novel antibacterial targets. Indeed, we identified homologous *yefM-yoeB* systems in a large number of bacteria including major pathogens. We aim to design peptides capable of interfering with the YefM-YoeB interaction, thus releasing the toxin to execute its detrimental function. For this purpose, we identified a short linear determinant within YefM that is involved in YoeB interaction. This peptide motif will be optimized for development of antibacterial lead molecules.

P-799

A meccano set approach of joining trpzip a water soluble β-hairpin peptide with a didehydrophenylalanine containing hydrophobic helical peptide

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A 16 residues long, water soluble, monomeric β-hairpin peptide "trpzip" [Cochran et al (2001) *PNAS* 98, 5578-5583], stabilized by tryptophan zipper has been linked via a tetraglycyl linker to a hydrophobic didehydrophenylalnine (ΔF) containing helical octapeptide. Circular dichroism studies of this 28 residues long peptide, "trpzipalpha" (Ac-GEWTWDDATKTWTWTE-GGGG-ΔFALΔFALΔFA-NH_2) in water have revealed the presence of both the β-hairpin and the helical conformations. This is the first instance where a ΔF containing peptide has been found to display a helical fold in water. The fluorescence emission wavelengths of Tryptophan in Ac-G-W-G-NH_2, trpzip and trpzipalpha were 341.5, 332.8 and 332.6 nm respectively. The fluorescence quantum yield of trpzip was 2.6 fold higher than trpzipalpha suggesting that proximal interactions between the β-hairpin and the helix caused the quenching of tryptophan fluorescence in the former by the ΔFs in the latter. The molar ellipticity of the far UV couplet characteristic of trpzip was reduced in trpzipalpha and the CD based thermal melting temperatures at 228 nm were 62°C (trpzip) and 57°C (trpzipalpha). A concentration dependent variable temperature CD study in water showed that in trpzipalpha, increasing temperature is detrimental to the β-hairpin, but it augments the helical motif by intermolecular oligomerization. Our results show that in water, trpzipalpha exhibits long-range interactions between two different secondary structures.

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The babab supersecondary structure of the Rossmann fold exhibits characteristics of a molten globule

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The Rossmann fold, which consists of two βαβαβ motifs related by a rough two-fold axis, is one of the most populated αβ folds. The elementary βαβαβ motif itself is a widespread supersecondary arrangement, and the βαβ submotif is present in nearly all α/β proteins. Therefore, the βαβαβ motif is often taken as an independent folding unit, and the βαβ submotif as a potential folding nucleus.
Here, we report molecular dynamics simulations, circular dichroism and NMR analyses on βαβ and βαβαβ from porcine lactate dehydrogenase to evaluate their intrinsic stability.
We show that the βαβ motif is not stable in solution and is not a folding nucleus. We also show that the βαβαβ supersecondary structure, despite its appearance of a functional and structural unit, is not an independent and thermodynamically stable folding unit. Rather, we provide evidence that the isolated βαβαβ motif folds as a molten globule since it retains most native secondary structures but very little tertiary structure.

P-801

A genetic algorithm for finding multiple low energy conformations under the 3D HP protein model

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The prediction of the 3D structure of a protein is one of the most challenging problems of molecular biophysics with important consequences for several areas of the molecular biology (*e.g.*, structural genomics, protein engineering). Moreover, the search methodology used to explore the protein's conformational space is a key aspect in this challenge. In this work we developed a search strategy, based on a genetic algorithm (GA), to find optimal multiple minima conformations on a simplified 3D hydrophobic-polar (HP) protein model. The method is based on a crowding population replacement to maintain conformational diversity and an adaptive approach is used to define the probabilities of the six operators used. New conformations compete with the most similar on the previous population. In the replacement strategy the hydrophobic core similarity is used as comparison criterion. The GA was tested against the most challenging sequences presented in the literature with 48-136 monomers. Equal or lower previously described energies were found and the multiple minima method allowed the GA to simultaneously find multiple distinct low energy solutions. The adaptive approach was effective because exploration of different positions on the conformational space requires different operators at different stages of the optimization process. The method proved robust and we expect that some of its features will be very valuable when dealing with complex molecular protein models.

P-803

Raman Optical activity of (4R) and (4S)-(L)-hydroxyproline and of acetyl-(L)-proline-glycine-(L)-pr

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Raman Optical Activity (ROA) is the difference in intensity of right and left circularly polarized Raman light scattered by chiral molecules. The first measurements of ROA were accomplished by Barron et al. in 1973, followed 2 years later by the recording of entire ROA spectra by Hug et al. In 2001 offset-free spectra could be recorded through the development by Hug of the simultaneous measurement of the molecule of interest and its virtual enantiomer. We present here for the first time the ROA spectra from 200 to 1900 $cm^{-1}$of 4R and 4S (L)-hydroxyproline ((4R) and (4S)-OH-P), acetyl-(L)-proline (Ac-P) and acetyl-(L)-proline-glycine-(L)-proline (Ac-PGP). The stereochemical hydroxylation of C4 on Pro introduces a second chiral center. In (4R)-OH-P (trans-OH-P), the isomer found in collagen, the OH and COOH group are on opposite sides of the pyrrolidine ring. (4S)-OH-P (cis-OH-P) is a proline antagonist in collagen synthesis. Ac-PGP, among other peptides, is released upon alkaline hydrolysis of corneal proteins in the alkali-injured eye (Kenyon et al. 1979). As glycine is the only amino acid which is not optically active, we could expect the ROA spectrum of Ac-PGP to be composed of Ac-P plus additional conformational information. The structure in solution is analyzed and compared to the NMR analysis published by Lee et al. in 2001.

P-804

Insights into the three-dimensional characteristics of HCA hydrophobic clusters

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Hydrophobic Cluster Analysis (HCA) allows the identification of reliable hallmarks of regular secondary structures (α-helix or β−strand) of globular proteins. This method is based on an essential feature of protein folding, hydrophobic compacity, and identifies various classes of hydrophobic clusters constituting structural "words".
Insights into the three-dimensional properties of HCA clusters have been gained through Voronoï tessellations, which we have developed for protein 3D structure analysis. As HCA clusters mainly correspond to the internal faces of regular secondary structures, we wished to further analyse their 3D environments. In this context, protein folded core can be described as a compact assembly of these HCA clusters.
With the Voronoï cells characteristics associated with each hydrophobic amino acid of HCA clusters, like their number of edges per face, their number of faces and their surfaces, it is possible to analyse the interactions between hydrophobic clusters. We have highlighted some geometric and assembly characteristics for a lot of cluster types, within a dictionary of 105 clusters preferentially associated with one of the two regular secondary structures.
The link between sequence information and characteristics of the 3D neighbourhood of clusters in their major regular secondary structure states open new perspectives for protein fold prediction from the sequence information alone.

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Structural studies on pore-forming peptides

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The resistance of pathological microorganisms to conventional antibiotic drugs has created a need for new antibacterial agents. Biologically active antimicrobial peptides that act as primary defense agents in a large variety of species are thought to have the potential as precursors for a new range of drugs from antibiotics to cancer treatments. This study has attempted to analyse the structural properties of membrane peptides and proteins through the use of model systems that have been designed to mimic their natural counterparts: We have successfully synthesised model membrane peptides with a beta-sheet structural motif and have used a wide range of techniques to analyse their interactions with phospholipid (PC) membranes.
The synthetic peptides were very hydrophobic and only soluble in fluorinated alcohols such as HFIP and to a lesser extent TFE. We found HFIP to have a very strong affinity for PC membranes and carried out a series of experiments to investigate this affinity.[1] Binding of HFIP to PC membranes was found to be reversible and we exploited this property in 2D crystal trials of our synthetic peptides. We over expressed the C-terminal domain of BrKA, a gram negative autotransporter protein, which forms a beta-barrel channel in the outer membrane (OMP), for comparison with our model peptides. We performed 2D crystal trials on the OMP and imaged the resulting protein arrays by STEM and AFM.

1. Sue M. Ennaceur and John M. Sanderson, *Langmuir*, 2005, **21**, 552-561

P-806

Conformational changes of eye lens proteins studied by combined SAXS and high pressure

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α-, β- and γ-crystallins are the main components of vertebrate eye lenses, with exceptional structural and associative properties. The crystallins are known to be exceptionally stable *in vivo* since they have to last the lifetime of the organism. They therefore represent an extreme case of stability versus unfolding and aggregation.
These proteins are mainly beta strands. γ-crystallins are 21 kDa monomers (from 50 to 80% sequence identity), and α-crystallins are large hetero-oligomers of about 800kDa. α-crystallins are molecular chaperone; they belong to the ubiquitous superfamily of small heat shock proteins, sHSPs.
Here, the conformation and the stability of α- and γ-crystallins were investigated by small angle X-ray scattering (SAXS) and high pressure, depending upon temperature and pH. At room temperature, α-crystallins have shown a partially reversible change in size from 2 to 3kb, and this effect was enhanced by the combination of temperature and pressure. In the case of γ-crystallins and in the pressure range up to 2kb, the pressure was combined with temperature and pH. The results depend upon the different γ itself.

P-807

Unfolding for binding – the talin-vinculin interaction

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The protein talin plays a key role in coupling the integrin cell adhesion molecules to the actin cytoskeleton and in integrin activation. The globular head of talin, which binds β-integrins, is linked to a rod containing an actin-binding site and binding sites for the protein vinculin, which regulates the dynamic properties of cell-matrix junctions. We have determined the structure of three domains which contain vinculin binding sites (VBSs) and shown that each of these are made up of helical bundles. The structures of complexes between vinculin and peptides corresponding to the VBSs show that the residues which interact with vinculin are buried in the hydrophobic core of the helical bundles of the talin domains. NMR studies of the interaction of one of these domains with vinculin shows that it involves a major structural change in the talin fragment, including unfolding of one of its four helices, to make the VBS accessible. While the observation of folding of unstructured regions of a protein on interaction with a 'partner' is quite common, this kind of major unfolding to permit a protein-protein interaction is much less common. Ways in which it may be regulated will be discussed.

P-808

Effects of branched beta- carbon dehydro - residues on peptide conformation

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Dehydro-amino acid residues have been shown to be strong inducers of folded conformations and can be used to design specific peptide structures. The systematic studies with various dehydro–residues have been carried out and a set of design rules have been developed. The limited investigations have indicated that the branched β–carbon dehydro–residues introduce steric constraints in peptides that differ significantly from those of ΔPhe and other non-branched β–carbon residues. The structure of a dipeptide N-Ac-Pro-ΔVal-$NHCH_3$ showed the formation of a type III β-turn conformation in contrast to a type II structure induced by ΔPhe at (i+2) position. In view of this systematic studies have been carried out with ΔVal and ΔIle by placing them at various sites and different combinations. It shows ΔVal and ΔIle at (i+1) position favored a type II conformation. Furthermore, the structure of tetrapeptide Cbz-ΔVal-Val-ΔPhe-Ile-OCH3 with ΔVal at (i+1) and ΔPhe at (i+3) positions resulted in a highly distorted 3_{10} –helical conformation indicating a complex behavior of the combined effects of ΔVal and ΔPhe residues. It has been further observed that the single branched β-carbon amino acid at (i+1) position in a peptide forms type II β-turn and at (i+2) position favors type III β–turn while in combination with ΔPhe it gives a 3_{10}-helical conformation.

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Unraveling the physical origin of the structure of fully denatured ubiquitin

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The structure and dynamics characterization of non-native states of proteins is crucial for understanding the mechanism of protein folding. Recently many experimental studies have shown variations of conformational propensity and flexibility along the backbone chain of fully denatured proteins. It has been supposed that areas of residual structure may serve as initiation sites of protein folding. However, the physical origin of these variations is still unclear.
We analyze the structure of fully urea-denatured ubiquitin. The experimental verification of conformational propensities of protein backbone is obtained through structurally dependent NMR parameters. Although the secondary structure of ubiquitin under strong denatured conditions is not detectable and no correlation with the native overall topology is found, the variations of NMR parameters along the backbone follow the secondary structure elements of its native state. We show that these variations are in accord with the recently developed electrostatic screening model of denatured proteins (1). In this model, the backbone conformations of residues in unfolded protein are determined by local backbone electrostatic interactions and their screening by backbone solvation.
1. F. Avbelj & R.L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **100**, (2003) 5742-5747.

P-810

Self-assembly of natural somatostatin into liquid crystalline nanofibrils

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The natural neuropeptide Somatostatin-14 is a cyclic tetradecapeptide hormone, with broad inhibitory effects on both endocrine and exocrine secretions. We report the self-assembly of somatostatin in solution, into stable liquid crystalline nanofibrils, based on the neuropeptide bioactive backbone conformation. The system was studied as a function of peptide concentration, milieu composition and time, using optical and electron microscopy, X-ray scattering, vibrational spectroscopy and SEC/RP-HPLC. In pure water, the formation of twisted nanofibrils (around 10nm wide and a few microns long) was characterized. Their structure relies on the native somatostatin β-hairpin and on intermolecular antiparallel β-sheets networks. The nanofibrils were observed to laterally associate further with increased concentration and time, as well as to generate hexagonal phases. Increase in ionic strength (sodium chloride, phosphate) was found to significantly favor the self-association process. The soft conditions of formation of the somatostatin nanofibrils support biological relevance, for instance to the biological mechanism of storage of the neuropeptide hormone.

P-811

Domain II of ribosome recycling factor is required for disassembly of the post-termination complex

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Ribosome recycling factor (RRF) consists of two domains and, in concert with elongation factor EF-G, triggers dissociation of the post-termination ribosomal complex. However, the exact function of the individual domains of RRF remains unclear. To clarify this, two RRF chimeras, *Eco*DI/*Tte*DII and *Tte*DI/*Eco*DII, were created by exchanging the RRF domains between the proteins from *Escherichia coli* and *Thermoanaerobacter tengcongensis*. The ribosome recycling activity of the RRF chimeras was compared with their wild-type RRFs by using *in vivo* and *in vitro* activity assays. The experiments show that like wild-type *Tte*RRF, the *Eco*DI/*Tte*DII chimera fails to complement the RRF^{ts} phenotype of *E.coli LJ14* (*frr*^{ts}) strain and has no polysome breakdown activity. However, under the same conditions, the *Tte*DI/*Eco*DII chimera complements the RRF^{ts} phenotype and has polysome breakdown activity equivalent to that of wild-type *Eco*RRF. The results indicate that domain II of RRF is the functional domain that is mainly responsible for the disassembly of the post-termination ribosomal complex, and the specific interaction between RRF and EF-G on the ribosomes mainly depends on the interaction between domain II of RRF and EF-G; while domain I of RRF is the main contributor for binding ribosomes and maintaining the stability of the RRF molecule. This study provides direct genetic and biochemical evidence for the assignment of individual functions of RRF domains.

P-812

A Powerful Driving Force in Protein Folding: Large Gain in Translational Entropy of Water

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A protein spontaneously folds into a unique native structure in physiological conditions. This process accompanies a huge loss of the conformational entropy (CE). Our major concern is to specify the factor that can compete with the CE loss. The previous discussions concerning protein folding have been focused on contributions to the free energy of folding from the interaction potentials in a system. A view lacking in earlier studies is that the folding is critically influenced by the translational movement of water molecules.
When solute particles contact each other in a solvent, the excluded volumes for the solvent molecules overlap, and the total volume available to their translational movement increases. This leads to a gain in the translational entropy (TE) of the solvent. This type of TE effect should be much stronger in protein folding where the tight packing of the side chains occurs.
An elaborate statistical-mechanical theory is employed to analyze the TE of water in which a peptide or a protein molecule is immersed. It is shown that the TE gain upon folding is large enough to compete with the CE loss. When water is replaced by another solvent whose molecular size is larger, the TE gain decreases to a remarkable extent. We suggest that the entropic loss accompanying the self-assembly and the formation of ordered structures in a living system is compensated mostly by the TE gain of water, highlighting an aspect of the crucial importance of water in sustaining life.

Posters
– Protein Folding –

P-813

A possible novel method of protein structure prediction; Origami Method

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Mechanism of protein folding has been mysterious since Anfinsen's dogma was sugested.Here
I would like to propose a possible novel method of protein structure prediction; Origami method.
The method comes from a protein backbone property of fluctuation and the residue hydrophobicity.

P-814

A spectrometric point of view on dimerization of bovine and porcine beta-lactoglobulins

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Electron spray ionization mass spectroscopy (ESI-MS) is a powerful tool for the investigation of the protein folding or proteins non-covalent interactions in solution since charge state distributions (CSDs) in ESI-MS are affected by the conformational state and mass relates on the association state. We used this tecnique to inquire at different pH and different conditions the dimerization process of the porcine and bovine β-lactoglobulins that share a high sequences similarity and close 3D structures. Dimerization of β-lactoglobulins is reversible and involves both electrostatic and hydrophobic interactions.
It was possible to detect simultaneously both the monomeric and dimeric form of the proteins in solution, pointing out the different dimerization behaviour of the two isoforms. We assessed the maximal stability of the dimeric structure at pH 4 for the porcine protein and pH 6 for the bovine one. Moreover we showed that bovine lactoglobulin has a stronger dissociation costant than the porcine protein. Further we showed that it is possible to modulate the dimerization equilibrium of the bovine isoform at pH 6 both increasing temperature and adding methanol without inducing denaturation of the protein.

P-815

Thermal and functional properties of E.coli outer membrane protein-receptor FhuA

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FhuA is *E.coli* outer membrane protein, which transports iron into the bacterial cell and also serves as receptor for several phages. In order to get more deep information about structural properties of FhuA, we've studied its thermal properties by means of calorimetry. We've also investigated the interaction between *T5*, *IRA* phages and directly *FhuA* by means of viscometry. The calorimetric result of heat denaturation of membrane protein *FhuA* and next deconvolution of the recorded calorimetric curve with two transitions has shown that in a chosen conditions the structure of *FhuA* consists of 4 domains. Though both *T5* and *IRA* phages grow on the common bacterial strain (*E.coli K12 HO830*), expressing *FhuA* the results of viscometric investigation show that under direct interaction of phages with *FhuA* the receptor activity of protein revealed only for *T5* phage. Therefore, we conclude that other than *FhuA* protein serves as receptor for *IRA* phage. It should be mentioned that the phage DNA ejection process induced by receptor was observed for the first time by us in an incessant regime.

P-816

Folding of parallel beta-sheet proteins associated with bacterial pathogenesis

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Many virulence factors from gram-negative bacteria are autotransporter proteins. The final step of autotransporter secretion is C→N-terminal threading through the outer membrane (OM), followed by folding. This process requires neither ATP hydrolysis nor a proton gradient. Pertactin, an autotransporter from *Bordetella pertussis* and the largest β-helix structure solved to date, folds much more slowly than expected based on size and native state topology, yet folding intermediates are not aggregation-prone. Equilibrium denaturation results in the formation of a partially folded structure, a stable core comprising the C-terminal half of the protein. Examination of the pertactin crystal structure does not reveal the origin of the enhanced C-terminal stability. Yet sequence analysis reveals that, despite size and sequence diversity, all autotransporters are predicted to fold into parallel β-helices, suggesting this structure may be important for secretion. For example, slow folding *in vivo* could prevent premature folding of in the periplasm prior to the assembly of the OM porin. Moreover, extra stability in the C-terminal rungs of the β-helix may serve as a template for the formation of the native protein during secretion, and formation of the growing template may contribute to the energy-independent translocation mechanism. Coupled with the sequence analysis, these results suggest a general mechanism for autotransporter secretion.

Posters
– Protein Folding –

P-817

Effect of Soft Confinement Formed by Cubic Lipid Phases on Protein Stability and Unfolding Behaviour

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Lipidic cubic phases formed by distinct water and lipid volumes provide bicontinuous 3D bilayer matrices that have specific and controllable water channel sizes and large surface areas. These systems have proven to be also valuable as membrane mimetic structures, as promising matrices for controlled-release and delivery of proteins, vitamins and small drugs in pharmacological applications, and they offer a 3D lipid matrix for successful crystallization of membrane proteins which do not easily crystallize in bulk solution. The present study is directed towards a better understanding of the interplay between curved cubic lipid phases and the protein entrapped within their aqueous channel structures. As model systems, we have chosen a cubic Ia3d phase formed by an uncharged lipid, monoolein, and incorporated different proteins, such as cytochrome c, α-chymotrypsin and insulin, in its narrow water channels. We show that the protein secondary structure and unfolding behaviour may be influenced by the confinement and, vice versa, the topology of the lipid matrix may change as a function of protein size and concentration. In fact, even new cubic lipid structures may be formed that are not known in pure lipid systems. Furthermore, we compare the aggregation scenario of insulin in bulk solution and in the narrow water channels of the cubic lipid matrix and discuss the differences found in terms of the geometrical limitations imposed by the confinement.

P-818

Thermal disruption of a spanning network of hydration water and conformational changes of elastin

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Hydration water strongly influences the structural and dynamical properties of biomolecules. The existence of a spanning hydrogen-bonded network formed by the hydration water enables the function of biomolecules at low hydration levels. We can expect that the formation/disruption of the spanning network formed by the hydration water in solution also affects crucially the protein properties.
We present the first computer simulation study of the thermal disruption of a spanning network formed by the hydration water of a biomolecule (elastin-like peptide). This process obeys the laws of 2D percolation transition, similarly to the formation of a spanning water network with increasing hydration level [1]. The spanning water network transforms into an ensemble of small water clusters with increasing temperature: it is still permanent at 280 K and exists with probability $\sim$50% at 320 K. In the same temperature interval, the conformation of the peptide changes noticeably: its radius of gyration increases sharply (by 15%) at about 295 K. These two phenomena may be related to the "inverse temperature transition" at about 310 K, where an elastin solution separates into two phases. In our simulations, the displacement of hydration water by the addition of a denaturant (urea) or by other peptide molecules causes an even stronger increase of the radius of gyration (up to 25%).
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P-819

Probing the folding capacity and residual structures in 79- and 110-residue fragments of staphylococcal nuclease

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N-terminal fragments of staphylococcal nuclease (SNase) with different chain lengths were used as a model system in the folding study. The detailed characterization of conformational states of 1-79 and 1-110 residues SNase fragments (SNase79 and SNase110) and their V66W and G88W mutants can provide valuable information on the development of conformations in the folding of SNase fragments of increasing chain lengths in *vitro*. In this study, the presence of retained capacity for folding and residual structures in SNase79 and SNase110 is detected by CD, fluorescence, FTIR, and NMR spectroscopy. SNase79 is represented as an ensemble of interconverting conformations. The fluctuating nascent helix- and â-sheet-like structures, localized in regions of A58-A69 and T13-V39, respectively are transiently populated in SNase79. The native-like tertiary conformations are obtained for G88W110 and V66W110 and for SNase110 in the presence of 2.0 M TMAO. Analysis of the results of such studies indicate that folding of SNase fragments is dominated by developing the local and non-local nucleation sites from native-like secondary structures and by intensifying the long-range interactions of residues at nucleation sites with residues further removed in sequence.

P-820

New amyloid-forming proteins

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Amyloid fibrils are formed by proteins or their peptides in the result of a conformational transition from alpha helix into beta-sheet structure. Despite the different nature of proteins-precursors their amyloids have common properties: beta-pleated sheet structure with individual beta-sheets oriented parallel to the main axis; insolubility *in vivo*; specific binding to Congo red and thyoflavin-T. Amyloid deposits are observed in different diseases such as myositis, myocarditis, cardiomyopathies and others. We showed that sarcomeric cytoskeletal proteins of titin family (X-, C-, H-proteins) of rabbit skeletal muscles are capable to form amyloid fibrils *in vitro*. These proteins already contain $\sim$90% of beta-sheet structure necessary for formation of amyloids. The amyloid nature of their fibrils was confirmed by electron, polarization and fluorescence microscopy. As X-, C-, H-proteins form amyloid fibrils easily *in vitro*, there is a danger of fast growth their amyloid deposits *in vivo*. Taking into account common properties of amyloids formed by different proteins, our results clear the ways for conducting by amyloidogenesis in human organs and tissues.
Work is supported by RFBR grants 03-04-48487, "Universities of Russia" 11.01.462 and Program of the Presidium RAS "Fundamental Sciences for Medicine".

Posters
– Protein Folding –

P-820-B

Effect of pressure on the conformation of proteins. A molecular dynamics simulation of lysozyme

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The effect of pressure on the structure and dynamics of lisozyme was studied by MD computer simulation at 1bar (101 325 Pa) and 3 kbar using GROMACS package. All-atoms (ff2gmx) force field were used for the minimization process and for all the MD simulation and kept all protein bond lengths constrained (LINCS algorithm). Water molecules (SPC/E model) were constrained using the SETTLE algorithm. For the electrostatic forces we applied the Reaction Field method. Lennard-Jones interactions were calculated within a cut-off radius of 1.4nm. The results have good agreement with the available experimental data, allowing the analysis of other features of the effect of pressure on the protein solution. The studies of mobility show that although the general mobility is restricted under pressure this is not true for some particular residues. From the analysis of secondary structures along the trajectories it is observed that the conformation under pressure is more stable, suggesting that pressure acts as a 'conformer selector' on the protein. The difference in Solvent Accessed Surface (SAS) with pressure shows a clear inversion of the hydrophilic/hydrophobic SAS ratio, which consequently shows that the hydrophobic interaction is considerably weaker under high hydrostatic pressure conditions.

P-821

Metal binding in amyloids beta peptides shows both intra- and inter-peptide modes

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XAS spectroscopy results show that there are two different structures of the metal binding site in the $A\beta_{1-40}$ peptide according to whether they are complexed with Cu^{+2} or Zn^{+2} ions. While the geometry around copper is suggestive of an intra-peptide binding with three histidine residues bound to the metal, the zinc site geometry is compatible with an inter-peptide aggregation mode. This result reinforces the hypothesis that assigns opposite physiological roles to the two metals, with zinc favouring and copper blocking peptides aggregation and consequent plaque formation.

P-822

Generalized-ensemble simulations of helical peptides

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In complex systems with many degrees of freedom such as peptides and proteins there exist a huge number of local-minimum-energy state. One way to overcome this multiple-minima problem is to perform a simulation in a generalized ensemble where each state is weighted by a non-Boltzmann probability weight factor so that a random walk in potential energy space may be realized (for reviews, see Refs. [1-3]). Three of the most well-known generalized-ensemble algorithms are perhaps multicanonical algorithm (MUCA), simulated tempering (ST) and replica-exchange method (REM). Recently, new generalized-ensemble algorithms which combine the merits of MUCA or ST and REM have been developed [4-6].In this poster, we present the results of these new generalized-ensemble simulations of small biomolecules.

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P-823

Interaction of the diphtheria toxin translocation domain with membranes: role of its N-terminal part

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Diphtheria toxin is a three domain protein: a catalytic domain, a translocation domain (T domain) and a receptor binding domain. After endocytosis, *i.e.* at acidic pH, the T domain inserts in the membrane of the target cell and helps the translocation of the catalytic domain into the cytoplasm. Therefore, the T domain has a key role in the strategy of internalization of the toxin.
The study of the interaction of the T domain with membranes and its pH dependence is important for a better understanding of the diphtheria toxin translocation mechanism. At least, two steps can be distinguished during the membrane insertion of the T domain. The first step involves hydrophobic interactions with the membrane and is related to the pH-induced stabilization in a molten-globule state. In the second step, electrostatic interactions are preponderant and the pH-sensitivity comes from changes of the balance between repulsive and attractive electrostatic interactions. The role of the N-terminal part of the T domain in the second step has been investigated by studying peptides corresponding to the amphiphilic helices found in this part of the domain. The results are correlated with those obtained with a single Trp mutant probing the N-Terminal region of the whole domain. The translocation mechanism will be discussed in view of the physico-chemical properties of the peptides.

Posters
– Protein Folding –

P-824

Intermediate states of formin binding protein WW domain: explored by replica-exchange simulation

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WW domain of formin-binding protein (FBP) is a model system for beta strand folding study. Although it is small, only 37 amino residues in total, the folding kinetics of FBP WW domain proved to be biphasic. An extensive molecular dynamics- Monte Carlo hybrid method, called replica-exchange simulation strategy is employed to study the folding/unfolding of the FBP WW domain in explicit water model. Begin with randomly chosen conformations from high temperature unfolding trajectory distributed in 88 replicas (88 different temperatures covering from 290K to 570K), the simulation lasts 30 nanoseconds in each replica. In the end an interesting distribution of conformations shapes up. We find that there are three distinctive subgroups, one being the unfolded conformations with RMSD averaging around 10Å , one being native conformations with RMSD $\approx$ 2.5Å, more interestingly, the third group having RMSD$\approx$5Å, an intermediate folding ensemble. By checking the intermediate ensemble in detail, we find that it is quite heterogeneous and the heterogeneity mainly comes from the flexibility of the C-terminal loop region. Our findings provide a microscopic picture of folding kinetics of the WW domain: the stable intermediate states with mis-registered hydrogen bonds on the C-terminal beta strand make this peptide folding as a three-state folding model rather than a usual two-state model.

P-825

Magnesium promotes conformational switching of Ca^{2+} sensor

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The importance of Mg^{2+}, one of the most abundant metal ion in the cell cytoplasm, relating to the calcium sensor mechanism is demonstrated. In this study it has been shown that the 134 amino acid long calcium binding protein from *Entamoeba histolytica* (*Eh*CaBP) can exist in three different forms namely, the calcium-free (*apo*) form, the magnesium bound (*apo-Mg*) form and the calcium bound (*holo*) form. These three forms have been characterized using chromatographic, calorimetric as well as various spectroscopic techniques. There is a radical difference in the stability between the calcium free and Ca^{2+} bound forms. The calcium free form has molten globule like characteristics. Mg^{2+} stabilizes the closed conformation of the *apo* form, where the hydrophobic core remains buried. The presence of Mg^{2+} significantly alters the calcium binding cooperativity thereby increasing the cooperativity of the conformational switching between the open and closed conformation which is an important aspect of such regulatory proteins. A structural model for the molten-globular form of *apo-Eh*CaBP and its equilibrium folding towards completely folded *holo* state in presence and absence of Mg^{2+}will be presented.

P-826

Identification and mutagenesis of a region of TnT required for the stability of TnT-TnI coiled-coil

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Evolutionarily conserved Heptad Hydrophobic Repeat (HR) domains present in troponin subunits TnT and TnI are involved in alpha helical coiled-coil formation. Using recombinant peptides from fast-skeletal TnT and TnI, we examined the contributions of amino acid residues within these HR domains as well as flanking these domains, to the stability of the coiled-coil interaction. A series of TnT fragments were tested for their ability to form coiled-coil with TnI HR domain. We show that the TnT region 166-178, although remains outside of the coiled-coil domain, is absolutely required for the stability of the coiled-coil. Interestingly, the region TnT 166-178 contains few absolutely conserved residues that are potential candidate for ionic interaction, as predicted by molecular modeling. Using single point mutants we show that among all the conserved residues, residue $Lysine_{175}$ is most important in stabilizing the coiled-coil interaction, whereas others play accessory role. We propose that the $Lysine_{175}$ initiates the stabilization of the coiled-coil interaction and then the other residues acts in a zipper like fashion.

P-827

Direct observation of mini-protein folding using fluorescence correlation spectroscopy

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The "Trp-cage" motif represents the smallest and one of the fastest folding mini-proteins known to date. The globular fold is characterized by a hydrophobic core burying a single tryptophan (Trp) residue. Here, we report on the direct observation of Trp-cage folding kinetics using fluorescence correlation spectroscopy (FCS).
Our method is based on the selective fluorescence quenching of oxazine dyes by Trp which becomes efficient only upon contact formation between the dye and the indole moiety of Trp. By site-specifically labeling the dye to Trp-cage, temporal fluorescence fluctuations of the dye-peptide conjugate, caused by intramolecular contact formation between dye and Trp, directly report on conformational dynamics and folding transitions of the peptide chain. In order to measure fluorescence fluctuations directly in solution we used FCS on a confocal fluorescence microscope setup. FCS allows us to reveal conformational dynamics with nanosecond time-resolution, under thermodynamic equilibrium conditions, and in highly dilute solutions (i.e. at nano-molar sample concentrations). Our method confirms microsecond folding kinetics of the Trp-cage motif, previously estimated with non-equilibrium temperature-jump techniques. We further investigated stability and folding rates under denaturing conditions and at various temperatures, giving further insight into structural transitions during the folding process.

Posters
– Protein Folding –

P-828

Peptide conformational search using Generalized Simulated Annealing method

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The three-dimensional structure of proteins is mainly determined by the sequence of amino acids, making possible the development of *ab initio* methods for peptides and protein structure prediction. We proposed a stochastic method based on a classical Force Field and the Generalized Simulated Annealing (GSA), which utilizes Tsallis generalization of Boltzmann-Gibbs statistics. We have applied this method for peptide conformational search and as a complement for Comparative Modeling of proteins, searching the conformation for loops and mismatched sequence alignments. The GSA efficiency depends on the right choice of the parameters involved in the conformational calculation: the qV parameter which defines a function for visiting the molecular energy surface; and the qA parameter defining an acceptance probability, both according with Tsallis Statistics. To avoid the conformational trapping in local energy minima we introduced a new parameter in this work, qT, to control the temperature decreasing. To investigate the qV, qA and qT best parameters set, we used the 18-alanine and 26-alanine peptides, which have a known alpha-helical structure in low dielectric environment. The global minimum energy occurs for the alpha-helix folded structure, and was found for qV ranging from 1.1 to 1.9, qA from 1.6 to 2.9, and qT from 1.1 to 2.4. We observed also that convergence values for qV decrease while for qA and qT increase for folded structures.

P-829

Monitoring the membrane interaction of diphtheria toxin T domain N-terminus with a single Trp mutant

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During the intoxication of a cell, the diphtheria toxin binds to a cell surface receptor, is internalized and reaches the endosome. The translocation (T) domain from the toxin interacts with the membrane of the endosome in response to the acidic pH found in this compartment. This process drives then the passage of the catalytic domain of the toxin through the membrane into the cytoplasm. The interaction of the T domain with the membrane involves at least two steps. In the first step occurring at pH 6, T adopts a molten globule conformation, which is able to bind superficially to the membrane through hydrophobic interactions by the C-terminal region (helices TH8 and TH9). In a second step occurring between pH 6 and 4, penetration into the membrane involves electrostatic interactions. This step leads to a functional inserted state.
Trp 281 was mutated to Phe in order to use Trp 206 located in helix TH1 as a probe of its behavior during the interaction with the membrane as a function of pH. We found that the second step is correlated with the reorganization of the N terminal region in the membrane and is controlled by electrostatic interactions.

P-830

TSE soil fate : conformational changes of prions induced by adsorption on clays studied by FTIR

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Studying the mechanism of retention of ovine prion protein in soils will tackle the environmental aspect of potential dissemination of scrapie infectious agent. The conformational transition from the monomeric cellular prion protein PrP^{C} in α-helical structure into the aggregated β-sheet-rich multimer PrP^{SC} is supposed to be responsible for the so-called prion diseases. It is commonly admitted that the recombinant PrP could serve as model of conversion of the normal prion protein PrP^{C}. Fundamentally, the interaction of proteins with surfaces either fluid or solid involves both protein binding and unfolding. Our goal in studying protein adsorption is to determine the nature and the amplitude of the structural changes occurring during non-specific adsorption. The protein-clay interaction depends on several parameters such as protein hydration, net charge, charge distribution on the protein surface. FTIR spectroscopy is well-suited to probe structural changes of proteins at a molecular level at aqueous/solid interfaces. The conformational states of the full-length ovine PrP adsorbed on the electronegative clay surface are compared to its solvated state in deuterated buffer in the pD range 3.5-9, using FTIR spectroscopy.

P-831

Kinetic characterization of the PrP oligomerization process reveals multiple pathways of assembly

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In pathologies due to protein misassembly, low oligomeric states of the misfolded proteins rather than large aggregates play an important biological role. To get better insight into the molecular mechanisms of PrPc/PrPsc conversion, we studied the kinetic pathway of heat induced amyloidogenesis of the full length recombinant ovine PrP (ARQ) at pH 4.0. According to the size exclusion chromatography experiments, three sets of oligomers were generated from the partial unfolding of the monomer. The effect of concentration on the oligomerization kinetics was different for the three species obsreved suggesting that they are generated from distinct kinetic pathways. Limited proteolysis and peptide analysis of the two best separated peaks showed a difference in the accessibility of the C-terminal domain of these two oligomers, and allowed the identification of regions undergoing a structural change during the conversion process. The analysis of correlations between oligomer populations as well as numerical kinetic modeling led us to propose a multi-step kinetic pathway describing the evolution of each species as a function of time.
The existence of at least three distinct oligomerization pathways on one hand and the differences in the accessibility of the two purified oligomers on the other hand reflect the structural plasticity of the PrP protein.

Posters
– Protein Folding –

P-832

On the three-dimensional information of a protein sequence

V. A. Risso, L. G. Gebhard, R. G. Ferreyra, J. Santos, M. E. Noguera, M. R. Ermacora
Universidad Nacional de Quilmes CONICET

In this work, βlactamase of *B. licheniformis* (ESβL) was used as an experimental model to (a) study the conversion of sequential information into 3D structure and (b) to investigate the distribution of conformational information in the polypeptide chain. By a novel approach, over thirty connectivity variants of the polypeptide chain were prepared; witch were also Nterminally truncated to a variable degree. The variants produced in *E. clil* were purified to homogeneity, refolded, and its structure content analyzed by circular dichroism, hydrodynamic behaviour and aggregation state. Several variants were dimeric in solution, suggesting a possible general inespecific stabilization mechanism. Most variants were compact and had different degrees of secondary and tertiary structure. A strikingly large number of variants showed native like spectroscopic signatures and significant enzymatic activity, which means that the very elaborate active site of beta lactamase is formed, at least in fractions of the molecules, despite the absence of long stretches of sequence. These findings are discussed in the light of the current knowledge of the protein folding process.
VAR and LGG contributed equally to this work.

P-833

On the three-dimensional information of a protein sequence

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VAR and LGG contributed equally to this work.

P-834

In situ formation of silk protein nano-particles studied by small angle X-ray scattering

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Silk threads from the Mulberry silkworm *bombyx mori* are used for the production of textiles since centuries. In modern applications silk proteins are chosen because of their good tensile strength, their high biocompatibility as well as of the resorbability for the human body. However, the processing of silk by the silkworm is barely understood. The silk proteins are stored as a solution in the glands of the silkworm and processed during the spinning into a co-block polymer like fiber. In a first step the random coil silk proteins are transformed into molecules with beta-sheet subdomains, which provide a protein-protein interface for the fiber assembly. This transformation can be mimicked by a rheometer applying a shear force to the silk protein solution. Applying combined small/wide angle X-ray scattering (SAXS/WAXS) transient formed silk nanoparticles upon increasing shear force were found. Further details of these macromolecules were derived by fixing the transient state with chemicals such as polyethylene oxide (PEO). The resulting data can be analyzed in detail by SAXS data evaluation software and low resolution models of the found nanoparticles were derived. Moreover, the internal structure of the particles was explored as well as suggestions for the silk processing of the silkworm could be made.

P-835

Giant Glossoscolex Paulistus Hemoglobin (HbGp)-Cethyltrimethylammonium Chloride (CTAC) Interaction

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Extracellular HbGp is a giant hemoglobin, similar to other annelid hemoglobins, having a molecular weigth of 3.1 MDa. The effect of CTAC in the oligomeric protein structure was assessed by optical absorption and emission spectroscopies. Optical absorption spectra of HbGp 0.075 mg/ml as a function of CTAC concentration from 0 up to 10.5 mM evidentiate changes both in Soret band at 414 nm, and at 490 nm associated to light scattering which appears at low CTAC concentration. Below 1 mM of surfactant extensive light scattering occurs together with significant shifts of λ_{max} of Soret band from 414 nm to around 400 nm, which is probably due to oxidation of the original oxyHbGp. Light scattering reaches a maximum value disappearing for higher CTAC concentrations. Fluorescence spectra show a significant increase in intensity (30fold) upon titration with CTAC. This is consistent with the dissociation of the oligomer with significant reduction of intrinsic quenching of tryptophan fluorescence due to the heme groups. Similar data were obtained at protein concentration of 3 mg/ml. In this case a significant increase of light scattering is observed with protein precipitation at a narrow CTAC range followed by re-disolution at higher CTAC concentration. Differently from anionic SDS surfactant, cationic one induces protein aggregation. Support: CNPq and FAPESP.

Posters
– Protein Folding –

P-836

Remodelling the folding of thioredoxin by removal of the C-terminal helix

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E. coli thioredoxin (TRX) is a monomeric α/β protein of 108 amino acids with a fold characterized by a central beta sheet surrounded by alpha helices. Two subdomains are topologically noticeable, but it is unclear whether their folding occurs in a concerted fashion. Subdomain TRX1-73 has been extensively studied as a model of a partially folded, with no tertiary or persistent secondary structure. This work describes the expression and characterization -by circular dichroism (CD), fluorescence emission, size exclusion chromatography, chemical cross-linking and light scattering- of a novel engineered fragment (TRX1-93) lacking the last stretch of 15 amino acids. After refolding from inclusion bodies, TRX1-93 shows a strong propensity to form soluble oligomers endowed with distinctive optical properties unlike those observed for the full protein. Although TRX1-93 also shows significant changes in secondary structure, Trp residues appear to occupy rigid and apolar environments. These findings support the existence of an alternatively folded form for TRX1-93. In addition, the secondary structure content of chemically synthesized peptide TRX94-108 and its ability to complement fragment TRX 1-93 upon refolding were also evaluated by CD. Taken together, the data herein presented shed light upon issues such as the distribution of information content relevant for folding along the polypeptide chain in regard to conformational stability.
With grants from ANPCyT, UBACyT and CONICET.

P-836-B

Aggregation processes in beta-amyloid peptides: effects of molecular chaperons

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Several neurodegenerative pathologies, like Parkinson's, Hungtington's and Alzheimer's diseases, are related to the formation of small peptides aggregates, which amyloid fibrils originate from. Understanding the molecular mechanisms responsible for these processes can, therefore, contribute to clarify the origin and, hopefully, to control the development of the afore mentioned diseases. Here we report the results of an *in vitro* study aiming to affect the aggregation kinetic of 1-42 and 1-40 beta-amyloid peptides by means of an endogenous chaperone-like protein (alpha-crystallin) and an exogenous polycyclic aromatic pigment (hypericin) that can perturb the aggregation process through stacking interactions with the peptides aromatic residues. Because of the well known problems in getting reproducible and reliable results, particular attention has been devoted to carefully check the preparation procedures of the samples. The effects of both alpha-crystallin and hypericin on the self-assembly process have been examined at different times of the aggregation kinetics. The results are discussed in relation with the involvement of different molecular structures in the amyloid fibrillation phenomenon.

P-837

Aggregation-prone intermediate protein structures on the refolding pathway

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The folding of the polypeptide chain into a native conformation can be studied by experimental systems, where the environmental parameters causing the denatured state can be easily and fast eliminated. One of such parameters is the high hydrostatic pressure. Refolding of the unfolded protein can be studied after decompression. The refolding pathway can contain several intermediate states.
On the other hand, the destabilization of the native proteins can populate conformations, where the polypeptide chain is not completely folded. These metastable conformations can easily aggregate. Deposition of insoluble protein aggregates plays a crucial role in the conformational diseases (Parkinson, Alzheimer's disease, amyloidozis, etc.)
Stability and conformation of the above mentioned metastable conformations were investigated in case of myoglobin, apo-horseradish peroxidase, lipoxygenase. Fluorescence and infrared spectroscopy and light scattering experiments were used to explore not only the structure but also the aggregation affinity of the intermediates
In case of all the above mentioned proteins a well defined temperature range could be determined, where the metastable not completely folded structures were populated considerably during the refolding process. These intermediate conformations were significantly more aggregation prone, than the native conformers existing in the same temperature range.

P-838

Unfolding of the extrinsic proteins of photosystem II (33kDa, 23kDa and 17 kDa) induced by pressure

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Unfolding of the three extrinsic proteins of spinach photosystem II induced by pressure has been systematically investigated. Thermodynamic equilibrium studies indicated that these proteins are very sensitive to pressure. At 20^{o}C all the proteins show a reversible unfolding transition by about 180 MPa for 33kDa, 200Mpa for 23kDa and 270 Mpa for 17kDa. The pH and temperature dependence of pressure unfolding of these proteins were explored. The stabilization effect of reagents sucrose etc on the proteins was found to associate not only with the increase in the unfolding free energy, but also with the reduction of the absolute value of ΔV_u. Pressure-jump studies of unfolding of 23kDa protein revealed a negative activation volume for unfolding and a positive activation volume for refolding, indicating that in terms of system volume, the transition state lies between the folded and unfolded states. Comparison of temperature dependence of $\Delta V_u{}^{\#}$, $\Delta V_f{}^{\#}$ and ΔV_u indicated that the thermal expansivities of the transition state and the unfolded state are similar and larger than that of the folded state.

Posters
– Protein Folding –

P-839

Association of subunits is a prerequisite for formation of the native structure of the dimeric IPMDH

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To answer the question whether subunits are autonomous folding units, or their association at an early stage of folding is required for formation of the native protein structure, denaturation-renaturation experiments were carried out with the dimeric isopropylmalate-dehydrogenase (IPMDH). Denaturation was induced by guanidine hydrochloride, renaturation was initiated by dilution and followed by activity measurements and fluorimetry. Reactivation is a complex process with an initial lag phase, indicating the presence of an inactive intermediate. The kinetics of the process is independent of protein concentration, suggesting that association of the two polypeptide chains takes place much faster than the rate limiting first order isomerisation step(s). Restoration of protein fluorescence during renaturation is also protein concentration independent, biphasic process, however the initial lag phase is replaced by an even faster burst increase of fluorescence. The first step leads to formation of an intermediate with a native-like fluorescence spectrum. Based on our experiments the following mechanism is proposed for refolding of IPMDH: $D+D \rightarrow I_2 \rightarrow I_2^* \rightarrow N_2$, where D is denaturated monomer, I_2 and I_2^* are inactive dimer intermediates, N_2 is native dimer, that means initial association of the polypeptide chains during refolding is a prerequisite for formation of the native 3 dimensional structure of IPMDH.

P-840

Exploring the free energy landscape of a folded protein by means of AFM stretching experiments

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The aim of our work is to study the mechanically induced folding and refolding of single proteins by means of an Atomic Force Microscope (AFM). The resulting data will be analyzed with theoretical methods, both to determine the folding pathway and to gain information on the energy landscape of real systems. Recently, experiments employing atomic force microscopy have shown that mechanical and thermal unfolding share several common features. We are using an AFM to perform mechanical stretching experiments on single biomolecules. The experiments that can be performed are particularly well-suited to reconstruct the folding-unfolding pathways as well as the free energy landscape of the examined protein. In particular we are interested in the free energy profile associated to titin and elastin, by considering a periodic loading of the AFM cantilever, instead of the usual linear ramp, and measuring the force as a function of displacement. These experiments will be complemented by theoretical and numerical studies. Molecular dynamics simulation of simple models but including the experimental geometry, will allow to examine in detail the effect of different experimental procedures (periodic loading versus linear ramp) proposed to reproduce equilibrium energy landscapes. Moreover, we will investigate the limit of applicability of the Jarzynski's equality which has been claimed to be able to be used to extract equilibrium results from non equilibrium measurements.

P-841

Free-energy landscape of the villin headpiece in an all-atom force field

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Ab-initio protein structure prediction and the elucidation of the mechanism of the folding process are among the most important problems of biophysical chemistry. Investigations of the protein landscape may offer insights into the folding funnel and help elucidate folding mechanism and kinetics.
We investigate the landscape of the internal free-energy of the 36 amino acid villin headpiece with a modified basin hopping method in the all atom forcefield PFF01, which was previously used to fold several helical proteins with atomic resolution. We identify near native conformations of the protein as the global optimum of the force field. More than half of the twenty best simulations started from random initial conditions converge to the folding funnel of the native conformation, but several competing low-energy metastable conformations were observed. From 76,000 independently generated conformations we derived a decoy tree which illustrates the topological structure of the entire low-energy part of the free energy landscape and characterizes the ensemble of metastable conformations. These emerge as similar in secondary structure content, but differ in the tertiary arrangement.

P-842

Thermal aggregation of two "beta-protein" models at different pH values

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The structural stability of proteins strongly depends on the environment and the lost of this stability may trigger a partial unfolding, leading in turn to the formation of aggregates. Such processes have been extensively studied also in view of their biotechnological and medical implications. In fact a large number of diseases is associated with protein misfolding and aggregation. Conformational changes play a keyrole in the aggregation processes and have their onset under particular external conditions. The aggregation pathways and the topology of the obtained supramolecular structures sizeably depend on the details of the involved conformational changes, which are determined by the details of the external conditions.
Here we present an experimental study on thermal aggregation processes of two model proteins mainly composed from β structures: β-lactoglobulin and concanavalin A, at different pH values.
The conformational changes of the proteins (whose association state depends by pH) and the aggregation pathways were monitored by intrinsic and suitable external dyes fluorescence. At the same time, the growth of supramolecular structures was followed by measuring the Rayleigh scattering of the excitation light. Secondary structure changes were followed by Circular Dichroism measurements. The results show that at different pH values the aggregation processes of both proteins follow different pathways determined by the variations in the native structure and by the details of the involved conformational changes.

Posters
– Protein Folding –

P-843

The protein circular dichroism data bank (PCDDB): A bioinformatics and spectroscopic resource

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We describe the development and creation of the Protein Circular Dichroism Data Bank (PCDDB), a deposition data bank for validated circular dichroism spectra of biomacromolecules. Its aim is to provide a resource for the biological and bioinformatics communities, by providing open access and archiving facilities for circular dichroism spectra. It is named by parallel with the Protein Data Bank (PDB), a long-existing valuable reference data bank for protein crystal structures. It will permits spectral deposition via user-friendly web forms and will include automatic reading of a range of data formats and data mining from file headers to facilitate the process. It will be linked, in the case of proteins whose crystal structures and sequences are known, to the appropriate PDB and sequence data band files, respectively. A series of validation tools that will provide reports on data quality are included (and are accessible as stand-alone software). It is anticipated that this data bank will provide readily-accessible biophysical catalogue of information on folded proteins that may be of value in structural genomics programs, for quality assurance and archiving in industrial and academic labs, as a resource for programs developing spectroscopic structural analysis methods, and in bioinformatics studies.

P-844

Study on multiple unfolding trajectories of GB1 by molecular dynamics simulations under the physical property space

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The 9 independent thermal unfolding simulations of GB1 have been performed. 12 physical property parameters of protein structure were chosen to construct a 12-dimension physical property space. Then the 12- dimension property space was reduced to 3-dimensions principle component property space. Under the property space, the unfolding pathway ensemble of GB1 was obtained. The pathway ensemble likes a funnel that was gradually emanative from the native state ensemble to the unfolded state ensemble. The unfolding trajectories have the similar variable trend during the native state and the transition state ensemble. During the unfolded state, the 9 unfolding trajectohries were divided into two types that one includes only one trajectory and the other include 8 trajectories. The first type of unfolded state was a discontinuity step distribution model, which is not random distribution. The second type of unfolded state was a near ellipsoid distribution model and a near random. There were substantial overlaps of unfolded state, indicating that thermal unfolded state consists of a confined set of property values that makes the number of unfolded state of protein to be much smaller than that was believed before.

P-845

Predictive all-atom protein folding with stochastic optimization methods

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The prediction of protein tertiary structure, in particular based on sequence information alone, remains one of the outstanding problems in biophysical chemistry. According to the thermodynamic hypothesis, the native conformation of a protein can be predicted as the global optimum of its free energy surface with stochastic optimization methods orders of magnitude faster than by direct simulation of the folding process.
We have recently developed an all-atom free energy forcefield (PFF01) which implements a minimal thermodynamic model based on physical interactions and an implict solvent model. We demonstrated that PFF01 stabilizes the native conformation of several helical proteins as the global optimum of its free energy surface. In addition we were able to reproducibly fold several helical proteins (the 20 amino acid (AA) trp-cage protein, the villin headpiece (36 AA), the conserved headpiece of the HIV accessory protein (40 AA), the headpiece of protein A (40 AA) and the 4-helix bacterial ribolsomal protein L20 (60 AA), as well as several beta-sheet peptides. We used several stochastic optimization methods: the stochastic tunneling method, an adapted version of parallel tempering, basin hopping techniques and distributed evolutionary optimization strategies. We discuss advantages and limitations of this approach to de-novo all-atom protein structure prediction.

P-846

DISCO : An optical biophysic beamline on SOLEIL Synchrotron

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We describe a new beamline for optical biophysic in construction on the synchrotron SOLEIL. The high briliance of the synchrotron beam, associated with its tunability on a broad part of the electromagnetic spectrum make it an excitation source of choice for several biophysical optical techniques. The DISCO beamline we present will consist of three endstations :
1. The **circular dichroism** (CD) endstation will benefit from the inclusion of the energies accessible in the VUV wavelength range (350-165nm) and from the natural polarization of the synchrotron beam. CD spectra of proteins covering a broad range of wavelenghts will enable better and finer structural analysis. Moreover, new biological chromophores such as sugars which absorb in the deep UV will be accessible in CD.
2. The **mass spectrometry** endstation,will benefit from an ionisation beam with even greater energies (down to 60 nm) comprising nebulisation at atmospheric pressure. Photoionisation of macromolecular bio-structures without any solvent restriction will produce perfect analytes for mass spectrometry,
3. The **multiparametric imaging** endstation, build on a confocal microscope, will use the great tunability of the synchrotron radiation (200-700 nm) to excite samples at many wavelenghts simultaneously. The temporal component of the beam will allow natural lifetime imaging by phase modulation - demodulation.

Posters

– Protein Folding –

P-847

Pressure perturbation calorimetic studies of solvation, unfolding and aggregation of proteins

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Pressure perturbation calorimetry (PPC) was used to study the solvation and volumetric properties of various proteins in their native and unfolded state. In PPC, the coefficient of thermal expansion of the partial specific volume of the protein is deduced from the heat consumed or produced after small isothermal pressure jumps, which strongly depends on the interaction of the protein with the solvent or cosolvent at the protein-solvent interface. The effects of pH and various chaotropic and kosmotropic cosolvents (glycerol, sucrose, urea, GuHCl, salts, etc.) on the solvation and unfolding behavior of the proteins was also investigated, and the observed volume and expansivity changes are correlated with further thermodynamic and spectroscopic properties of the systems. Depending on the type of cosolvent and its concentration, specific differences are found for the solvation properties of the proteins, and the volume change upon unfolding may even change sign. Taken together, the data obtained lead to a deeper understanding of the solvation process of proteins in different cosolvents in their native and unfolded states. In addition, the effects of confinement and crowding on the solvational properties of the proteins were studied. Finally, the use of PPC for studying intermolecular interactions and aggregation (amyloidogenesis) phenomena of proteins (e.g., insulin, PrP) will be discussed.

P-848

The relation of N-terminal residues and structural stability of L-chain apoferritin

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The denaturation of apoferritin by acidic solution was studied. Ferritin, the ubiquitous iron storage protein, represents a well known polymeric assembly that is highly resistant to chemical and physical denaturants. It is a cage-shaped protein which is composed of 24 subunits. Natural vertebrate ferritins are copolymers of two different subunits, L- and H-chains. In the recombinant H-chain apoferritin (rHF), the structural stability is decreased by deletion of the N-terminal residues.

We studied the effect of N-terminal residues of recombinant L-chain apoferritin (rLF) on the acidic pH denaturation and re-assembly. We constructed rLF and mutant rLFs which are lack of 4 (Fer4) or 8 (Fer8) amino acid residues from the N-terminus and investigated their stability by CD spectra. Among three, Fer8 has the least endurance against pH decrease. In the case of Fer8, the re-assembly of subunits into apoferritin can be performed by increasing solution pH without causing the by-product while huge aggregations are caused in Fer0 and Fer4. The structural comparison of three mutants indicates that the hydrogen bonds of inter- and intra-subunits decrease by the loss of the N-terminal residues. Therefore, it is elucidated that the hydrogen bonds of inter- and intra-subunits from N-terminal residues affect the molecule stability and re-assembly of L-chain apoferritin.

Posters

– Regulation of Membrane Transport –

P-850

Transport activity of the monocarboxylate transporter 1 is increased by carbonic anhydrase

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The enzyme carbonic anhydrase (CA), which catalyses the conversion of CO_2 and H_2O to bicarbonate and protons, is present in nearly all animal cells, and is highly expressed in astrocytes. It is known that CA can bind to several membrane transporters, forming a transport metabolon and thereby enhancing the transport activity of the protein. In this project we have studied the functional interaction of the enzyme with the monocarboxylate transporter 1 (MCT1), which transports lactate and other monocarboxylates together with protons and is believed to play a pivotal role in the metabolite shuttling between astrocytes and neurons. Therefore we expressed MCT1 and then injected CA into *Xenopus* oocytes. Our results indicate a direct binding of CA to the MCT1, leading to a CA-induced increase in acid/base flux mediated by the transporter. Interestingly, the effect was insensitive to the CA inhibitor ethoxyzolamide and to the nominal absence of CO_2/HCO_3^-, but disappeared when binding of CA to the MCT1 was hindered. It seems, that CA, bound to the MCT1, mediates local buffer capacity by removing protons transported into the cell via the MCT1. This helps to stabilise the proton gradient close to the cell membrane, and thereby enhances the transport activity of the MCT1. These findings suggest that CA can enhance metabolite-acid/base transport, by forming a transport metabolon with the MCT1.

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P-851

EPR studies of the flip-flop phenomenon in unsymmetrical bolaamphiphile medium-sized vesicles

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Bipolar lipids found in archaebacterial membranes, generally termed bolaamphiphiles, induce increased stability in membranes exposed to environments such as acidic conditions, high temperatures, high salt concentrations and/or absence of oxygen. We have synthesized a spin labeled unsymmetrical bolaamphiphile that self-organises in water solutions in multilamellar vesicles and shows slow flip-flop phenomenon in comparison to conventional liposomes. Generally, the flip-flop from the exovesicular to the endovesicular membrane surface is a relatively slow process, which is due to the high energy barrier in transferring the polar amphiphilic heads through the lipophilic membrane. It can be involved in membrane transport mechanisms and in facilitating the transport, cells have evolved to use various supramolecular strategies. The half-life of the flip-flop is estimated to more than twelve hours. We are now modulating the flip-flop rate by incorporating chemical modifications such as addition of cyclopentanes, double or triple bonds into the bridging chain of the molecule, in order to control the membrane transport via the flip-flop mechanism.

P-852

Influence of Lipid Membrane Composition on P-glycoprotein Activity

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P-glycoprotein (P-gp), a membrane ATPase expelling many structurally unrelated compounds, is one of the major contributors to multidrug resistance. It is proposed that substrates bind to it within the membrane and are exported from there out of the cell. P-gp substrates are generally hydrophobic and their binding to the transporter is governed by their ability to partition into the membrane. The intimate association of both P-gp and its substrates with the membrane suggests that P-gp function may be regulated by the composition of the lipid bilayer. As detergents influence the membrane properties and have been shown to affect P-gp ATPase activity, we developed virtually detergent-free proteoliposomes to investigate the influence of the membrane environment on the ATPase activity of P-gp. The basal and substrate induced ATPase activity was dependent on the cholesterol level of egg phosphatidylcholine (PC) membranes. The compound concentration at half maximal activation of P-gp (K_M) in proteoliposomes correlated with the affinity of the respective compound to liposomes consisting of the same lipids as the proteoliposomes tested. In conclusion, the basal and drug-induced ATPase activity of P-gp is strongly dependent on the cholesterol content in detergent-free P-gp/egg PC/cholesterol proteoliposomes.

P-853

The influence of semisynthetic derivatives of phenolic lipids on activity of yeast ABC pumps

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Phenolic lipids are the natural amphiphilic long-chain homologues of orcinol (1,3-dihydroksy-5-methylbenzene). They occur in numerous plants and microorganisms. Resorcinolic lipids exhibit high affinity for lipid bilayer and biological membranes and are able to modify the activity of membrane enzymes (e.g. PLA_2, AChE). The influence of semisynthetic derivatives of phenolic lipids on yeast PDR protein activity was studied by spectrofluorimetric method using the potentiometric fluorescence probe diS-C_3(3). The probe is expelled from *S. cerevisiae* by ABC pumps and can conveniently be used for studying their performance. Two pump-competent S. cerevisiae strains and different pump-free mutant strains were used to experiment to check the effect of the semisynthetic derivatives of phenolic lipids on activity of ABC transporters. Two of these derivatives, named 23.1 and 23.2, seem to affect the activity of PDR pumps. Their influence on activity of yeast plasma membrane multidrug resistance ABC pumps is concentration-dependent.

Posters

– Regulation of Membrane Transport –

P-854

Voltage dependence of processes related to electrogenic membrane transporters

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Electrogenic membrane transporters, such as the sodium-bicarbonate cotransporter (NBCe1), may induce dependence on membrane potential upon processes which are innately voltage-independent. We tested this hypothesis by heterologously co-expressing electrogenic NBCe1 from human kidney in oocytes of the frog *Xenopus laevis* with the electroneutral rat monocarboxylate transporter MCT1. The apparent intracellular buffer capacity was increased by NBCe1 expression and became voltage-dependent by 7 mM/10 mV membrane depolarisation. Lactate transport via the MCT1 not only became enhanced after co-expression with NBCe1, but also dependent upon membrane potential. Injection of carbonic anhydrase CAII from bovine erythrocytes into oocytes enhanced the efficacy of NBCe1 activity, identifying an additional, CA-sensitive, membrane current via NBCe1. Our results show that NBCe1 adds voltage-dependent buffer capacity to the cytosol; this is suggested to be the prime cause for enhancing acid/base-coupled transport and conferring membrane potential dependence on transporters which are stoichiometrically electroneutral. These interactions may have functional consequences for cells and tissues, where electrogenic and electroneutral processes interact, such as in brain, heart and muscle.

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P-855

Polyglycerol-Based Block Copolymers Accelerate Doxorubicin Permeation into MDR Tumour Cells

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Multidrug resistance (MDR) of tumours is associated with overexpression of the P-glycoprotein responsible for an active drug efflux from cells. Block copolymers of ethylene oxide and propylene oxide (Pluronics) are known to cause a pronounced chemosensitization of tumour cells. The effect may be due either to specific polymer – protein interactions or to unspecific lipid bilayer disturbance. We have shown recently that amphiphilic copolymers with various hydrophilic and hydrophobic blocks can disturb lipid bilayers. Importantly, that block copolymers of propylene oxide and glycerol (PPO-PG) with hyperbranched "corona" induced larger effects then Pluronics with linear polyethylene oxide chains. In the present work we have shown that PPO-PG copolymers increase DOX cytotoxicity towards human erythroleukemia (K562iS9, K562/DOX) and breast carcinoma (MCF7/DOX) resistant cell lines. Using confocal and two-photon microscopy, we demonstrated that these copolymers accelerated DOX penetration into resistant cells, inhibited efflux and caused drug redistribution into nuclei.
A clear correlation between the ability of the polymers to disturb lipid bilayers and favour drug accumulation in MDR cells was disclosed. This finding points to an unspecific mode of the copolymers' chemosensitizing activity.

P-856

Two-dimensional crystallization of co-reconstituted Ca2+-ATPase, phospholamban and sarcolipin

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The sarcoplasmic reticulum Ca^{2+}-ATPase and its regulators phospholamban (PLB) and sarcolipin (SLN) form a primary control mechanism in the recovery of resting state calcium levels in the myocardium. Defects in the regulation of Ca^{2+}-ATPase by PLB and SLN are central determinants in cardiac contractility and disease states such as cardiomyopathy. Given the significance of these proteins, the structural details of their regulatory mechanisms remain an important future goal for the clinical improvement of heart disease. Using co-reconstitution into proteoliposomes at low lipid-to-protein ratios, we have examined the effects of mutation on the functional properties of PLB and SLN, revealing novel insights into calcium pump regulation. In addition, these same co-reconstituted proteoliposomes have been used for structural studies by electron cryo-microscopy. In an attempt to better define the structural interactions between PLB, SLN and Ca^{2+}-ATPase, we have sought methods for the production of large two-dimensional crystals suitable for high resolution electron crystallography. We previously utilized the co-reconstituted proteoliposomes to produce long, tubular crystals suitable for helical reconstruction. Our new procedure comprises three steps – co-reconstitution, membrane fusion, and crystallization – producing large two-dimensional crystals suitable for high resolution structural studies. Herein, we will present our latest results characterizing the structural interaction between PLB, SLN and Ca^{2+}-ATPase.

P-857

New evidence concerning the role of helix IV in the function of Mel B symporter

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Melibiose permease of *E.coli* (Mel B) is a membrane bound ion-coupled sugar symporter that uses the favorable Na^+, Li^+, or H^+ electrochemical potential gradient to drive cell accumulation of α- or β- galactosides. Cysteine scanning mutagenesis, electrophysiological (SSM - solid supported membranes) and fluorometric measurements, were used in order to better understand the role of specific parts of the protein in the function of this symporter. The SSM technique combines a rapid solution exchange with the high sensitivity of planar lipid membranes. It employs a solid supported membrane as a capacitive electrode and allows the time resolved investigation of charge translocation during the catalytic cycle of such transporters as Na^+/solute symporters. In order to obtain some more precise information about the function of Mel B symporter, starting from the C-less MelB, the mutant G117C was constructed and from electrical, spectrofluorimetric and FRET measurements carried out on this mutant, in the absence and in the presence of specific inhibitors, conclusions were drawn about the possible role of the helix IV in the function of the symporter.

Posters

– Regulation of Membrane Transport –

P-858

How important is protein flexibility for transport through ion channels?

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Certain ion channels are selective for K+ over other ions, but the geometry of the pore does not explain selectivity because thermal fluctuations are too large. I extend the usual treatment of ion channels with molecular dynamics simulation by calculating the static and dynamic pair correlations between monovalent ions and ion channels (gramicidin-A and KcsA), and also between certain small, complex cations and the gramicidin channel. This means not only the radial-distribution functions or the density profiles, but also correlations between the ion and the mass and charge densities of various regions of the protein. The advantage of this approach is that it systematically identifies the elements of the protein and modes of motion that contribute to selectivity, and illustrates the decay of correlations.

Recently, Noskov et al. [1] showed that thermal fluctuations protect selectivity. My results on the interaction of ions with carbonyl groups agree with theirs, but take the analysis further to higher correlations. The key new element is the study of the time-correlation functions that describe the motion of the ions through the channel, borrowing methods originally developed for the study of dense or even supercooled liquids.

[1] Noskov, Berneche and Roux; Nature 431; p830; 14 October 2004.

P-859

Effects of nortriptyline and chlorpromazine on anthroylouabain-labeled Na,K-ATPase

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The effects of nortriptyline and chlorpromazine (CPZ) on the fluorescence properties of anthroylouabain (AO)-treated Na^+,K^+-ATPase of electrocyte membranes from *E. electricus* are studied. Na^+,K^+-ATPase oscillates between two major conformations E_1 and E_2 during ion transport cycle. The cardiotonic steroid ouabain specifically inhibits this enzyme binding to the E_2 conformation. The fluorescent label AO presents increased fluorescence when binding to the ouabain site of Na^+,K^+-ATPase. Tricyclic drugs such as the antipsychotic CPZ and the antidepressant nortriptyline inhibit Na^+,K^+-ATPase activity in the micromolar range. For the E_2 enzyme, but not E_1, nortriptyline was found to increase the fluorescence in a concentration dependent manner, suggesting a further stabilization of E_2. For both conformations, CPZ induces negligible fluorescence change up to 10 μM. The fluorescence of ATPase-bound AO, however, strongly increases upon ultraviolet exposure after CPZ treatment at concentrations around 20 μM. Fluorescent products of CPZ-photodegradation were studied in pure buffer and in the presence of membranes. The results suggest that CPZ binds to Na^+,K^+-ATPase and photolabels amino-acid residues near the ouabain binding site.

P-860

Conformational dynamics of a lactose proton symporter

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Transporter proteins are an essential class of proteins, catalysing the transfer of molecules across membranes. Increasing numbers of transporter structures are becoming available, opening the way to study their dynamic properties using computational techniques. We present a study on the conformational dynamics of the Lactose Proton symporter Lactose Permease using Molecular Dynamics Simulations. These simulations exhibit large scale conformational changes from the initial Intracellularly open conformation to a more closed conformation that may be significant to the transport mechanism. The conformational change is analysed to identify the contributing motions.

P-861

Origin and role of Na+/H+ exchanger activation in chemically-induced apoptosis

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pH_i-regulating transporters can be modulated by extracellular stimuli leading to pH_i changes that have been implicated in cell death. Thus, a few studies have shown that the Na^+/H^+ exchanger 1 (NHE1) is either down- or up-regulated following diverse apoptotic stimuli, resulting in either acidification or alkalinization (Lagadic-Gossmann et al., *Cell Death Differ*, 2004). However, the modulation and role of NHE1 in chemically-induced apoptosis are yet to be precisely determined. We have previously shown that the NHE1 activation observed upon exposure to benzo[a]pyrene (B[a]P 50 nM) is partly responsible for the related apoptosis (Huc et al, *Faseb J*, 2004). By using carboxy-SNARF-1 as pH-sensitive fluoroprobe and microspectrofluorimetry, we now show that NHE1 activation is due to Jun kinase (JNK) activation, resulting from reactive oxygen species (ROS) produced during metabolism of B(a)P and might involve lipid raft. When analysing B(a)P-induced apoptosis, we have found that cariporide significantly reduces both nuclear fragmentation and caspase-3 like activity. We further show that NHE1 activation and/or alkalinization affects the mitochondrial ROS production detected during the apoptotic cascade, likely via an effect on the complex III of the electron transport chain. Altogether, our results suggest that apoptotic xenobiotics, such as benzo[a]pyrene, induce an early activation of NHE1 that might play a significant role in the subsequent mitochondria-dependent apoptosis.

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– Regulation of Membrane Transport –

P-862

Molecular dynamics studies of a bacterial ATP-binding cassette transporter

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ATP-Binding Cassette (ABC) Transporters form an important superfamily of membrane proteins which couple ATP hydrolysis to the active transport of diverse compounds across the cell membrane. Their biomedical relevance is highlighted in examples such as multidrug resistance to antibacterial and anticancer agents, and cystic fibrosis. The availability of crystal structures of three complete bacterial ABC transporters provides an opportunity to study structure-function relationships at the atomic level. In this work, we carry out multi-nanosecond Molecular Dynamics simulations of the Vitamin B_{12} importer from *E. coli* (BtuCD), with both the complete multimeric transporter embedded in a phospholipid bilayer and the soluble subunits in a membrane-free environment, in an attempt to elucidate some of the conformational changes which arise during the transport event. ATP-bound and ATP-free structures are used to investigate the effect of nucleotide on the system. A range of analytical techniques have been applied to assess the dynamic behaviour of the protein during the simulations, which includes measurements of: conformational drift, residue flexibility, transmembrane domain (TMD) movement, concerted protein motions, nucleotide-binding and translocation pathway changes.

P-863

Lateral diffusion in tethered bilayer membranes

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Tethered bilayer membranes (t'BLM) provide a useful platform for the investigation of bilayer membranes as well as embedded membrane proteins. We have developed a modular system, which is suitable for surfaces providing gold and oxide surface coatings.
These systems serve as a quasi natural environment for the study of membrane proteins, being functionally incorporated into a lipid bilayer, which is covalently bound (tethered) to the substrates. Functionality could be shown using electrochemical methods.
Here we present a study of these systems, basically the membrane itself, using fluorescence recovery after photobleaching (FRAP) studies in order to investigate lateral motion in the lipid bilayers. Lateral mobility is essential for successful incorporation of large membrane protein complexes. We will present first results of experiments that try to differentiate motions hindered due to the tethering from diffusion in free floating or suspended bilayers.
The information gained in this study will serve for improvements in the chemical structure of the tethered molecules. We will develop the system as a basis for bio sensing applications, where embedded proteins will serve as actual sensing elements.

P-864

Effects of copper ions on the Escherichia coli growth and proton-potassium exchange

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Copper ions are required for the function of many important enzymes in *Escherichia coli* but can cause a number of toxic cellular effects also. It's interesting to reveal the influence of copper ions on the growth of bacteria and proton-coupled membrane systems. Upon transition of *E. coli* MC 4100 wild-type culture to stationary growth phase a decrease in redox potential (E_h) from the positive values ($\sim$ +140 mV) to the negative ones (of -380 to -550 mV), resulting H_2 production by formate hydrogenlyase (FHL) have been studied. Copper increased a latent growth phase duration as well as delayed a logarithmic growth phase in concentration-dependent manner. During the anaerobic growth, the production of H_2 was strongly inhibited in the presence of $CuCl_2$ (2 mM). 0.1 mM $CuCl_2$ was inhibited H_2 production under experimental conditions with glucose. The inhibitory effect of copper ions (0.1 mM) on *N',N'*-dicyclohexylcarbodiimide (DCC)-sensitive H^+/K^+ exchange was also observed: K^+ uptake was decreased and the stoichiometry of DCC-inhibited ion fluxes varied. Interestingly, these effects on H^+ and K^+ fluxes were absent for the mutant HD700 (*hyc*-operon for hydrogenase was deleted). We suggest that copper ions, inhibiting the activity of FHL, have an effect on H^+/K^+-exchanging mechanism which is the proton F_0F_1-ATPase associated with K^+ uptake Trk system. This effect may be due to the relationship of FHL with the ion-exchanging mechanism above under fermentation at alkaline pH.

P-865

SEIRAS as a method for the analysis of transport-mechanisms through membrane-proteins

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The surface-enhanced infrared absorption spectroscopy (SEIRAS) is used for the investigation of two membrane proteins, the Cytochrome c Oxidase (CcO) and the Bacteriorhodopsin (bR). Of central interest are the transport-mechanisms of electrons (CcO) and ions (bR).
The main parts of the setup are an infrared light source, a hemispherical Si-crystal, in which the beam is internal reflected and a Plexiglas cell with buffer solution (see schematic scetch). The beam is totally reflected on the inner flat surface of the crystal, but the evanescent wave excites surface plasmons in a chemically adsorbed gold-layer on the crystal (attenuated total reflection spectroscopy-ATR). The protein to be analysed is attached at the gold surface and can absorb certain wavelengths. The gold is need for the surface-enhancing effect.
CcO plays a major role in the respiratory chain, the retinal protein bR is a photosynthetic protein.
The CcO is immobilized on the gold surface via the affinity of its histidine-tag to a nickel-chelating nitrilo-triacetic acid (NTA) surface. For the bR we incorporate the protein in a lipid membrane, which is attached on the gold surface by the sulfuric bindings of 2,3-di-O-phytanyl-sn-glycerol-1-tetraethylene glycol-D,L-lipoic acid ester lipid (DPTL).
The sensitivity of this method is further enhanced by modulation of an external parameter, like the electric potential.

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– Regulation of Membrane Transport –

P-866

Formation of Lipid and Polymer nanotubes with Optical Tweezers

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We have developed a procedure to make lipid and polymer nanotubes of up to one cm long and 50 nm in diameter, from the surface of giant liposomes and polymersomes, using microfluidics and optical tweezers. The liposomes and polymersomes were formed, using elcetroformation method, from phospholipids and amphiphilic diblock copolymers, respectively. The polymer tubes were made extremely robust by cross-linking them using chemical reactions. We are currently studying the transport of molecules in the cross-linked nanotubes for use in nanofluidic networks.

P-867

Basal Ca2+ leak from endoplasmic reticulum of submandibular acinar cells

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Under the resting conditions Ca^{2+} concentration in agonist-sensitive Ca^{2+} stores reflects a balance between active uptake mediated by a Ca^{2+}-ATPase (SERCA) and passive efflux of Ca^{2+}. This Ca^{2+} leak appears to be a common property of Ca^{2+}-storing organelles, but the nature of the leak in submandibular acinar cells remains unclear. We have studied the Ca^{2+} leak pathways in the endoplasmic reticulum (ER) of acinar cells of rat submandibular salivary gland by directly measuring concentration Ca^{2+} in the ER ($[Ca^{2+}]_{ER}$) in mag-fura 2/AM preloaded cells while $[Ca^{2+}]_i$ was clamped at a resting level with a EGTA/Ca^{2+} mixture. We have shown that thapsigargin (Tg) or Ca^{2+}-free buffer treatment completely blocked Ca^{2+} uptake by SERCA after the first minute of superfusion and caused a Ca^{2+} leak represented by continuous decline in $[Ca^{2+}]_{ER}$. This Ca^{2+} leak from the ER was not sensitive to Tg, heparin and ruthenium red and therefore appears to be independent of the SERCA, the $InsP_3$ receptor and the ryanodine receptors. However, treatment with puromycin (0.1-1 mM) to remove nascent polypeptides from ER-ribosome translocon pores increased Ca^{2+} leak from the ER by a mechanism independent of the SERCA, $InsP_3$ or ryanodine receptors. Thus we conclude that basal Ca^{2+} leak from the ER of submandibular acinar cells occurs through translocon pores in the ER membrane.

P-868

Phenyltin chlorides transfer across the model lipid bilayer

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The well known group of organotin compounds that exhibit toxic properties in relation to the biological systems are phenyltins. No studies have been performed as yet to establish directly whether organotins such as diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT) cross the lipid bilayer. We have performed experiments that showed transfer of those compounds across the lipid bilayer using the stopped–flow technique and desorption of those compounds from a monolayer using the Langmuire technique. Obtained results demonstrate that DPhT and TPhT first adsorb onto the lipid bilayer surface, in diffusion controlled manner, within a very short time (0.05 s), whereas the membrane passing was observed in a minute's time range. The long time kinetics show a complex dependence on the kind of compound, its concentration and the presence of cholesterol in the membrane. The desorption of both compounds from the monolayer to water subphase occurs in a minute's time range. These observations may explain the known fact, that the influence of organic, amphiphilic tin (and also lead) compounds is more toxic than that of inorganic ones.The phenyltins much easier (compared with tin or lead ions) penetrate e.g. blood – brain barrier.

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P-869

Quantification of the release rate of proteins from liposomes

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An in-vitro method was designed to measure transmembrane transport rates. Liposomes were prepared by extrusion with dipalmitoyl phosphatidylcholine (DPPC) and optionally cholesterol, and loaded with a peptide (zinc-insulin tagged with a fluorescent group or BSA). After removal of the non-encapsulated peptide from the liposome solution by gel filtration, the release of the peptide from the liposomes was monitored by fluorescence as a function of time at various temperatures. The transport was greatly accelerated by the presence of a specific proprietary excipient molecule (cyclopentadecanolide – CPE215™), which effectively triggered the release of the peptide. A mathematical model was developed to quantify these results. A semi-empirical nonlinear equation involving four parameters fits the protein release profiles. Then a neural network predictions model was used to correlate the different release condition parameters and the four semi-empirical fitting parameters based on the experimental data sets. Most release data fit well with the mathematical model, further supporting our theory of a two step release mechanism.

Posters

– Regulation of Membrane Transport –

P-870

Dynamics of the full-length P-glycoprotein in the POPC bilayer

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The overexpression of P-glyoprotein (P-gp) is one of the major causes of multidrug resistance (MDR) in cancer chemotherapies. Many P-gp inhibitors have been designed to reverse the MDR effect, but the structure-activity relationships of the P-gp inhibitors and substrates still remain largely unknown. Until now, it is still very challenging to obtain the high-resolution P-gp structures and currently only low-resolution electron microscopy structure is available. This has caused the structure-based design of "P-gp-ignoring" therapeutic agents a remote goal.
However, the recent determination of X-ray crystal structures of bacterial lipid A transporter, MsbA, has provided eligible structure templates for homology modeling of P-gp. We have therefore conducted explicit solvent molecular dynamics simulations of the full-length efflux pump, human P-gp, in an excessively hydrated POPC bilayer to refine the homology model. Both free and ATP-bound forms of P-gp have been simulated. The entire system consists of more than 365,000 atoms. Our molecular dynamics simulations have shown that the overall architecture of P-gp remained very stable for tens of nanoseconds, while the observed membrane undulation was rather large. The simulation results have allowed us to investigate the conformational changes of P-gp upon ATP binding in the efflux process and to predict the possible binding site of various known substrates and inhibitors. The refined structure models of P-gp by our simulations could be used as the basis for further drug design.

P-871

New insights of hypericin blood transport and its incorporation into the plasma membrane

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Hypericin (Hyp) is one of the active compounds from *Hypericum perforatum* (an herb usually prescribed as antidepressant). The bioavailability of this hydrophobic molecule is a very important issue for medical applications. The goal of our work was the study of Hyp blood transport mechanisms. Techniques of absorbtion spectroscopy, electrophoresis and fluorescence microscopy were used in order to define the properties of Hyp-albumin and Hyp-lipoproteins complexes and to explain the action of Hyp at the plasma membrane level. Hyp bind to several electrophoretic (SDS-PAGE) bands evidenced by mice plasma migration. Both albumin and lipoproteins bind to Hyp, forming complexes, during the blood transport process. Different types of lipoproteins from males and females plasma mice were evidenced by gradient electrophoresis to bind Hyp. Hyp-albumin complex was also identified by absorbtion spectra, and the ratio A_{594}/A_{550} has a pH-dependence. Hyp interaction with plasma membranes was also examined on cell culture by fluorescence microscopy, and Hyp plasma incorporation is a dose- and incubating time-dependent process. Our results partially elucidate the plasma fractions that bind Hyp, contributing to its blood transport. This study proposes a new mechanism of Hyp cellular insertion, discussing its plasmatic membrane penetration due to its high hydrophobicity.

P-872

Interaction between the energy metabolism and externally applied electric fields in yeast cells

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Electric fields are often used for biophysical or biomedical treatment of biological cells, e.g. cell fusion or killing of cells. However, only a few data about the possible mechanisms of electrosensitivity of biological cells are available. Since electrostimulation always induces depolarization of biomembranes, an impact of the energy metabolism is obvious due to the regeneration of electrochemical gradients by the expenditure of cellular energy. Our aim is to investigate the interactions between externally applied electric fields and the aerobic/anaerobic energy metabolism of yeast cells. For this, we have constructed a new electrical interface for local stimulation of biological cells with variable duration and amplitude. When applying short lasting electrical pulses to yeast cells, we find a direct response of the energy metabolism (measured by NADH-fluorescence) to these pulses. A sudden and fast decrease in NADH is followed by a slower recovery of the fluorescence signal. These NADH-signals are abolished in the presence of Antimycin A or KCN, demonstrating the importance of mitochondrial energy production for this phenomenon. We attribute these changes to the immediate break down of ATP as a consequence of the regeneration of the membrane potential (ATPases) and the slower regeneration of ATP by mitochondrial respiration.

P-873

P-glycoprotein couples MgATP hydrolysis and intramembrane translocation of a fluorescent model drug

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P-glycoprotein (P-gp) is an active membrane transporter capable of expelling out of the cell a large number of potentially cytotoxic amphiphilic molecules with unrelated chemical structures. As a consequence, P-gp may be responsible for multidrug resistance of tumors against chemotherapy (MDR); it also plays a key role in absorption, biodisposition and elimination of many pharmaceuticals. To understand the molecular mechanisms of the transmembrane drug transport mediated by Pgp, it is highly desirable to design a convenient assay for measuring both P-gp ATPase activity and P-gp transport function. To do so, we used inside-out native membrane vesicles, prepared from MDR cells and containing high amounts of P-gp. We took advantage of the specific property of a fluorescent dye, the carbocyanin JC-1, known to be expelled out of MDR cells: above a critical concentration (the "CJC"), this dye forms J-aggregates which emit a fluorescence at a wavelength very different from that emitted by the monomer. In the presence of MgATP, the P-gp-containing vesicles accumulated JC-1, which exceeded locally the CJC and thus formed intraluminal J-aggregates; these aggregates allowed accumulated JC-1 both to be sequestered inside the vesicles, by dramatically slowing down its passive backdiffusion, and to be specifically detected. Kinetic characterization of this transport suggests that JC-1 is first translocated to the exoplasmic leaflet of the vesicle membrane before its internalization into the aqueous phase of the vesicle lumen.

Posters

– Regulation of Membrane Transport –

P-874

The transport mechanism of melibiose permease: a study using electrical measurements and fluorescence techniques

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The melibiose permease (MelB) of Escherichia coli is a membrane bound carrier that uses the favorable Na+, Li+, or H+ electrochemical potential gradient to drive cell accumulation of alfa-galactosides (melibiose, raffinose) or beta-galactosides (methyl-1-thio-beta-D-galactopyranoside). Electrophysiological techniques have proved to be extremely useful tools to investigate the mechanism of ion transfer across the membrane by ion-coupled transporters. Using a solid supported membrane (SSM) as a capacitive electrode a rapid solution exchange can be combined with the high sensitivity of planar lipid membranes and allows time resolved investigation of the charge translocation during the catalytic cycle of Na+/solute symporters. This technique has been combined with fluorescence measurements, which report on structural changes during the substrate transport process of the carrier. We have used time resolved tryptophane fluorescence, fluorescence energy transfer with a fluorescent sugar substrate and site specific fluorescence of a label attached to a cysteine residue on the protein. This allowed us to identify conformational transitions during the reaction cycle of the melibiose permease. We could assess their electrogenicity and determine rate constants. A kinetic model for Na+ and melibiose binding and transport is presented.

P-875

Inhibition of multidrug resistance-associated protein MRP1 and Kv channels by natural polyphenols

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Resistance to cytotoxic agents remains a major obstacle to successful chemotherapy in cancer. Best-characterized form of drug resistance is caused by the overexpression of genes encoding membrane drug pumps, like P-gp or MRP1. In present study, activity of several plant polyphenols (flavonoids and stilbene) against MRP1 has been studied using functional assay based on efflux of MRP1 fluorescent substrate. Very recently, a role of Kv1.3 potassium channels in proliferation of various cancer cells was suggested. In our study the effect of the plant polyphenols on voltage-gated potassium channels Kv1.3 was investigated by patch-clamp electrophysiological method. Some of studied compounds were found to be active inhibitors of multidrug resistance-associated protein MRP1 and voltage-gated potassium channels, and their properties are promising for further research in the field of anticancer activity of natural products.

P-876

Interaction of quinolones with bacterial porin OmpF: fluorescence quenching studies

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Quinolones are widely used antibiotics witch develop their antibacterial action by inhibition of important bacterial enzymes. Consequence of the internal location of their target of action, the translocation of this drugs trough the outer membrane is an essential step for their antibacterial action.
In vivo studies have been showing that OmpF is important for the entrance of some of these antibiotics, but the exact degree of involvement of this protein in the transport of the different members of this group of antibiotics, remains unknown.
In this study, the quenching of the intrinsic tryptophan fluorescence of OmpF, in presence and in the absence of the drugs and by two distinct quenchers, was used as a first approach, to elucidate ligand-induced structural changes and consequently prove the differential involvement of OmpF in the entrance of these antibiotics in the bacterial cell.
The results obtained reveal that the degree of interaction with the protein is related with the hydrophobicity of the different antibiotics. This kind of evidence suggests that the entry by the porin channel is not the only path used by these antibiotics and that it is more important for the latest generations of this group because of their increased hydrophilic characteristics.

P-877

Mechanisms of conductivity changes in a cell suspension due to cell electroporation

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Electroporation (EP) is a phenomenon where increased permeability of cells exposed to an external electric field is observed. The induced transmembrane voltage presumably leads to the formation of aqueous pores in the phospholipid bilayer, which increases permeability of the membrane for molecules and ions. EP is currently used in many biomedical applications including transfer of genes and electrochemotherapy of tumors. Still, the molecular mechanisms of the process are not fully explained. Recently it was proposed that EP could be monitored in real-time by measuring electric conductivity of tissue. So far the studies focused mostly on a single pulse, however in biomedical applications usually several pulses are used. In our study we used a train of electric pulses to analyse the relationship between electric conductivity and cell permeabilization. Current-voltage measurements during and after pulse application were performed in dense suspension of cells. Conductivity changes were analysed numerically using finite elements method and compared with the percentage of permeabilized cells. We obtained a transient increase in conductivity above a certain voltage with complete relaxation in < 1s. Substantial changes in conductivity are also due to the diffusion of ions through membrane pores and osmotic swelling. We further show that relation between conductivity and permeabilization level is indirect.

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– Regulation of Membrane Transport –

P-878

Interaction of various types of amphipols with the Ca2+-ATPase from sarcoplasmic reticulum

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Amphipols are amphipathic polymers designed to replace or supplement detergents in membrane protein solution studies. For the study of the Ca^{2+}-ATPase from sarcoplasmic reticulum, previous experiments have revealed both advantages and disadvantages to the use of a polyacrylate-based amphipol, A8-35. These issues have been reinvestigated using four different amphipols. Size exclusion chromatography showed that, although A8-35 aggregates in the presence of millimolar concentrations of calcium -an effect that probably accounts for most of the aggregation of ATPase/A8-35 complexes observed in our previous work-, aggregation can be avoided by resorting to a sulfonated version of A8-35. We also found that all amphipols tested slowed down the rate of calcium dissociation from its binding sites and reduced ATPase activity, while protecting the solubilized protein against denaturation. This suggests that association with the polymer may damp the protein's dynamics, perhaps due to the multipoint attachment of the polymer to its hydrophobic transmembrane surface. Such a "Gulliver" effect could contribute both to the protection of membrane proteins against denaturation and to the reversible inhibition of SERCA1a.

P-879

Electrophysiological characterization of mitochondrial uncoupling protein 2

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Electrophysiological characterization of the vast number of annotated channel and transport proteins in the postgenomic era would be greatly facilitated by the introduction of rapid and robust methods for the functional incorporation of membrane proteins into defined lipid bilayers. We present an automated method for reconstitution of membrane proteins into lipid bilayer membranes, that substantially reduces both the reconstitution time and the amount of protein required. We have applied this well-defined system to the characterization of a novel mitochondrial uncoupling protein, UCP2 and demonstrated that UCP2 exhibits protonophoric function exclusively in the presence of fatty acids, similar to that previously shown for its homologue UCP1. The membrane conductance was proportional to the concentration of the reconstituted UCP2 in presence of oleic acid or eicosatrienoic acid, and was inhibited by ATP.

P-880

The novel electrogenic K^+/ca^{2+} exchanger from human erythrocyte: dependence on mono and di-valents cations

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Human red blood cell (RBC) shows a life span of ∼120 days. Although little is known about the mechanism underlying the removal of senescent cells, it is widely accepted that a rise in internal Ca2+ plays a crucial role. Using the T.U.G.O. Patch Clamp Technique we had presented the first direct electrophysiological evidence of a mechanism capable to account for the Ca2+ entry during cell aging, a K+/Ca2+ exchanger. This current presents a sigmoid voltage-dependence (1). We present the capacity of K+/Ca2+ exchanger to transport mono (K+, Cs+, Rb+) and di-valent cations (Ca2+, Ba2+, Mg2+, Zn2+), all cations, except Cs+, produce the reversion of the current, suggesting that those ions were transported through this mechanism. Using different approximations we have established that K+ is 2.5 times more permeable than Rb+, and Cs+ seems to be impermeable. Ca2+ and Ba2+ seemed to show almost the same permeability, and Ca2+ is 850 times more permeable than Mg2+ and even more permeable than Zn2+. Under physiological conditions this exchanger seems to move Ca2+ into the cell. Hereby, we present new direct evidences, and a preliminary sequence of permeability for the novel electrogenic K+/Ca2+ exchanger in RBC. We already had suggested that this mechanism might be the principal responsible for the rise in internal Ca2+ during cell senescence.
(1) Romero, J.G., Romero, P.J. (2005) Biophysical J. 88(1): 593a

P-881

Orientational and conformational changes in transmembrane domains of membrane proteins

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Multidrug transporter proteins promote the active transmembrane efflux of drugs thereby decreasing their accumulation in the intracellular medium. Crystallisation of these resistance proteins is a way to gain deeper insight into the resistance mechanism. To overcome difficulties related to crystallization of membrane proteins, new experimental approaches have been developed to gain information on the structural changes involved in drug transport. We examine here and illustrate with examples how infrared and fluorescence spectroscopy can provide new insights into the structure and orientation of the membrane domains of transporters in particular and how ligand-protein interaction can affect the structure and orientation of transmembrane domains. We will also illustrate how such methods opens new possibilities for the detection of conformational changes that are transmitted from the cytosolic domains to the transmembrane domains and vice-versa. How lipid micro-domains modify the transmembrane structural parameters will be discussed through a few examples.
Vigano,L, Manciu and J-M Ruysschaert
Acc Chem Res. 2005 Feb;38(2):117-26. Review
Grimard V., Li C., Ramjeesingh M., Bear C.E., Goormaghtigh E., and Ruysschaert J.-M. J. Biol. Chem., 279(7):5528-5536 (2004)

Posters

– Regulation of Membrane Transport –

P-882

Model and biological membranes modification by some N-Oxides

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A series of new *N*-oxides of tertiary amines (NTA) was checked for its biological activity. Individual compounds differed in the length of substituted alkyl chain. The primary goal was to find if they can be used as effective antioxidants and, to what degree they modify used model (liposomes) and biological (erythrocytes, algae and cucumber) membranes. Various methods were used in order to do that. A mechanism of the interaction between NTA and membranes was studied by measuring their potency to hemolyse erythrocytes, to influence a phase transition temperature in DPPC liposomes, to change a membrane potential of algae cells. The measure of the interaction of NTA with cucumber cells were potassium leakage, chlorophyll content and inhibition of growth of hypocotyls.
Antioxidative abilities of NTA were determined by measuring their efficiency to protect erythrocytes against membrane lipid oxidation induced by UV irradiation and by comparising their antioxidative efficiencies with that of Trolox (vitamin E analogue) in chromogen experiments.
The work was sponsored by the Polish Research Committee (KBN), grant no. 2 P04G 088 27 and no. 3 T09B 075 27.

P-883

KB-R7943 effects on mechanics and energetics of rat myocardial bigeminies

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During bigeminies two fractions of extracellular Ca^{2+} can be distinguished, being one of them verapamil (VER)-sensitive. VER-insensitive Ca^{2+} fraction is lithium-sensitive, it is responsible for the postextrasystolic potentiation (PP) and probably related to the Na^{+}/Ca^{2+} exchanger (NCX). To further investigate NCX role in bigeminic contractions the effects of a specific NCX inhibitor (KBR 7943) at a concentration that affects the Ca^{2+} entry mode (5 μmol.l^{-1} (Satoh et al., 2000)) was studied. Mechanical and myothermic responses of extrasystolic (ES) and postextrasystolic (PES) contractions were studied. PP was calculated as the difference between pressure-time integral (PtI) of PES and regular contraction (RC). The heat released by RC, ES and PES could be decomposed into two components PtI-independent (H1 and H2), and a third one (H3) PtI-dependent (Ponce-Hornos et al., 1994). KBR affected both PtI_{ES} (1.1$\pm$0.1 mN.mm^{-2}.s, n=18, p<0.05) and PP (2.1$\pm$0.2 mN.mm^{-2}.s, n=18, p<0.05), as well as heat components, without affecting H3/PtI ratio, indicating that economy for pressure maintenance was not affected. The simultaneous decrease in PtI_{ES} and PP in the presence of KBR suggests that VER-insensitive (lithium-sensitive) fraction is KBR-sensitive, confirming its relation to the NCX working in the Ca^{2+} entry mode.
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P-884

Calorimetry and mechanics of Ca^{2+} transporting systems in rat myocardial bigeminies

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Bigeminic contractions (BC) (extrasystole (ES) and postextrasystole (PES)) were mechanical-energetically studied. Heat released was decomposed in three components, one (H3) mainly attributed to pressure developed. Neither muscle (active heat (Ha) over PtI_{BC}) nor contractile economies (H3/PtI) were affected by stimulation pattern. CAFFEINE decreased pressure-time integral (PtI) of PES (Δ:64.1$\pm$1%), postextrasystolic potentiation (PP=difference between PtI_{PES} and regular contraction) (Δ:79.3$\pm$2%) and PtI_{ES} (between 37.6$\pm$3% at extrasystolic interval (ESI) of 0.6s and 5$\pm$0.1% at ESI 0.3s), suggesting a central role for SR providing Ca^{2+} during PES, but a dependence on ESI's duration during ES. $(Ha/PtI)_{BC}$ increased with CAFFEINE at all ESIs (Δ:101$\pm$12%), due to a higher energetic cost of Ca^{2+} removal associated with a reduced capacity of SR to retain Ca^{2+}. VERAPAMIL decreased PtI_{ES} (Δ:43.4$\pm$3%) without effects on PP or H3/PtI, suggesting that one fraction of extracellular Ca^{2+} is affected by VERAPAMIL, while VERAPAMIL-insensitive fraction is responsible for PP. 45 mM Li and 5 μM KB-R7943 decreased both PtI_{ES} (Δ:71.3$\pm$2%) and PP (Δ: 67.3$\pm$3%) without affecting H3/PtI, suggesting an association of VERAPAMIL-insensitive fraction to the sarcolemmal Na-Ca exchanger. This Ca^{2+} would then be accumulated at the SR from the first ES assuring PP during bigeminies.
[Grants: CONICET PIP-02544/2003-4; UBA O023-2004-7.]

P-885

Voltage-dependent electrogenic Cl-/H+ exchange by the endosomal CLC proteins ClC-4 and ClC-5

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CLC proteins are found from prokaryotes to mammals. They function as plasma membrane chloride channels or provide neutralizing anion currents for V-type H^{+}-ATPases that acidify compartments of the endosomal/lysosomal pathway. Vesicular CLCs have been thought to be Cl^{-}-channels, in particular because ClC-4 and ClC-5 mediate plasma membrane Cl^{-}-currents upon heterologous expression. We have shown, however, that these two mainly endosomal CLC proteins rather function as electrogenic Cl^{-}/H^{+} exchangers, resembling the transport activity of the bacterial ClC-e1 that has been crystallized. Neutralization of a critical glutamate residue not only abolished the steep voltage-dependence of transport, but also eliminated the coupling of anion flux to proton counter-transport. ClC-4 and ClC-5 may still compensate the charge accumulation by endosomal proton pumps, but are expected to tightly couple vesicular pH-gradients to Cl^{-}-gradients.

Posters

– Regulation of Membrane Transport –

P-886

Evaluation of thermodynamical and biophysical properties of peritoneal membrane of bof. Bubalis

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The specific conductance's of aqueous solution of electrolytes (viz.NaF, NaCl, $NaNO_3$, $Na2SO_4$, KF, KCl, KNO_3, K_2SO_4, $MgCl_2$, $CaCl_2$, $FeCl_3$, $MnCl_2$,$CrCl_3$,$CuCl_2$, $CoCl_2$,)have been measured across Peritoneum at temperatures between(15-35)$^{\circ}$C.Conductance attains a maximum limiting value at higher concentrations for each electrolyte due to a progressive accumulation of ionic species within the transmembrane region. The membrane becomes more and more conductive to incoming ions and attaining a limiting value due to the fact that an electrically neutral pore, which is specific for a particular ion, is unlikely to contain more than one type of ion. Consequently, at high electrolyte concentration, the pore saturates and the conductance's approaches a limiting value. The values of specific conductance measured follow the sequence for anions; SO_4^{2-} > Cl^- > NO_3_ > F^-. Whereas for the cations the sequence is K^+ > Na^+; Ca^{2+} > Mn^{2+} > CO^{2+} > Cu^{2+} > Mg^{2+}; Cr^{3+} > Fe^{3+}. The energy of activation for the cations as well as for the anions follows the sequence (For cations): Ea $_K{}^+$> Ea $_{Na}{}^+$; Ea $_{Ca}{}^{2+}$> Ea $_{Mn}{}^{2+}$ > Ea $_{CO}{}^{2+}$ > Ea $_{Cu}{}^{2+}$ > Ea $_{Mg}{}^{2+}$; Ea $_{Cr}{}^{3+}$ > Ea $_{Fe}{}^{3+}$. (For anions): Ea $_{SO4}{}^{--}$ > Ea $_{Cl}{}^-$ > Ea $_{NO3}{}^-$ > Ea $_F$–. The linear plot between free energy change and membrane potential at different concentration across peritoneal membrane shows the validity of complex transport phenomenon. The values of ΔS+, ΔH^+, Ea. and ΔF+ were calculated.

P-887

Change of cells size under modification of ion-transport system of membrane

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Size of cell are related with ion-transport membrane processes. Some transport processes in membrane affect cellular size [1]. Currently the methods allow observing cellular size changes dynamics at high resolution are slightly developed. Here laser cytomonitoring – LC was used [2]. Technique of small-angle light scattering on suspended particles. LC makes it possible to obtain size distribution functions of particles and to observe their time evolution. Calcium ionophore – peptide A23187 was used. Density and volume distribution of cells were detected depending on calcium concentration. Problem of effectiveness of LC at erythrocytes investigation was examined. Resuspended in various environments erythrocytes were studied. Size distribution functions of erythrocytes were received according to ionophore A23187 concentration. Cellular size change kinetic was observed during 90 min after ionophore addition. Results were obtained to show sensitivity of laser cytomonitoring to erythrocytic volume changes. 1.Lang F, Busch GL, et al. // Physiol. Rev. 1998, 78, 247-306. 2..Shaitan KV, Lobkov AF, et al. // Biol. Membr. 2002, 19, 3, 210-218 This work was supported by RF MES (prs No 0431, 01.106.11.0001, 01.165.11.0001), RFBR (pr. No 04-04-49645).

P-888

Electrophysiologic study of AP in *Chara corallina* - Indication of its biochemical nature

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Recent experiments demonstrate that the concentration of Ca^{2+} in cytoplasm of *Chara corallina* internodal cells plays important role in electrical excitation of the plasma membrane. The concentration of free Ca^{2+} in the cytoplasm – $[Ca^{2+}]_c$ is also sensitive to visible light. Both phenomena were simultaneously studied by noninvasive measurement of action potential (AP) and magnetic field (with Superconducting QUantum Interference Device- SQUID magnetometer) in very close vicinity of electrically excited internodal *Chara corallina* cells. A temporal shift in the depolarization maximum, which progressively occurred after transferring cells from the dark into the light, can be explained by the extended Othmer model. Assuming that the change in membrane voltage during the depolarization part of AP is the direct consequence of an activation of $[Ca^{2+}]_c$ sensitive Cl^- channels, the model simulations compare well with the experimental data. We can say that we have an example of electrically elicited AP which is of biochemical nature. Electric and magnetic measurements are in good agreement.

P-889

Multidrug resistance in bacteria

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The mostly widely employed mechanism of drug extrusion in bacteria is via membrane transport proteins called efflux pumps. In Gram-negative bacteria, multidrug resistance is conferred by tripartite complexes, rather than by a single transport protein. Through these systems, a wide range of substrates is expelled from the cytoplasm, through the periplasmic region, to the exterior of the cell. Among these complexes, the AcrAB/TolC system in *Escherichia coli* is formed by an Inner Membrane efflux pump, AcrB, an Outer Membrane protein, TolC, and a periplasmic protein known as an adaptor, AcrA. The components of this complex are studied, in order to provide insights into drug transport in bacteria. Here we present a dynamics study on MexA, homologue of AcrA from *Pseudomonas aeruginosa*. The protein has been studied by Molecular Dynamics simulations in bulk water. A structural adjustment by the periplasmic protein is required in order to engage both the bottom part of the OM protein and the top region of IM protein. The dynamics on MexA reveals a flexible behaviour of the protein in water. The major concerted motions observed are the hinge-bending of the two domains, and the rotation of the β-barrel domain. These can be related to the adaptation of MexA (and AcrA) to the OM and IM proteins during the process of assembly in forming the complex, and during the opening of the channels.

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– Regulation of Membrane Transport –

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HYDROPHOBIC PHOTOLABELLING ANALYSIS OF LIPID-PROTEIN STOICHIOMETRY

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We have developed a method to assess the number of lipid association sites based on the use of a photoactivatable phosphatidylcholine (PC) analogue. [^{125}I]TID-PC/16. This reagent locates specifically within the phospholipidic membrane and reacts indiscriminately upon photolysis. It is therefore possible to directly analyze the interaction between the protein and lipids belonging to its immediate environment. We have studied the composition of the lipid annulus of three different membrane proteins belonging to the P-type ATPase family: sarcoplasmic reticulum calcium pump (SERCA), sodium pump and plasma membrane calcium pump (PMCA). The proteins were photolabelled in mixed micelles containing detergent, [^{125}I] TID-PC/16 and different amounts of PC. The stoichiometry was estimated after the extent of the labeling reaction had been independently assessed. Our results are in good agreement with the values previously obtained by EPR for SERCA and sodium pump. Lipid-protein stoichiometry measured on PMCA rendered 17 ± 2 PC molecules per protein monomer, a value which fits reasonably well with that predicted by geometrical considerations. This analytical development could find general application to the study of lipid-protein interactions.

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Membrane composition influences Pluronic-induced transport of antitumour drugs

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Copolymers of ethylene oxide (EO) and propylene oxide (PO) of $EO_{n/2}PO_mEO_{n/2}$ type (Pluronics) are able to reverse multidrug resistance of tumor cells. Obviously, first step of Pluronics action on cells is their interaction with cell plasma membranes. Previously we have shown that Pluronics bind to artificial membranes and enhance accumulation of anti-tumor drug doxorubicin inside pH-gradient liposomes and flip–flop of NBD-labeled phosphatidylethanolamine in liposomes composed from lecithin only. Taking into account different lipid composition of cell membranes it seemed relevant to evaluate its significance for membrane sensitivity to Pluronic. We made binary liposomes containing, besides the lecithin, different amounts of other lipids of natural origin (cholesterol, phosphatidylethanolamine, cardiolipin, ganglioside GM1, sphingomyelin, phosphatidic acid). A reverse dependence between the microviscosity of membranes and their sensitivity to Pluronic effects was demonstrated. These data may be important for the understanding of the mechanisms of Pluronic interaction with cells.

Posters

– Light Driven Systems –

P-892

Expression of channelrhodopsin-2 in mammalian cells

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Photosensory responses of green algae are mediated by rhodopsins with microbial-type chromophores.The recently detected cDNA sequences from the green alga *Chlamydomonas reinhardtii* that encode microbial opsin-related proteins were termed Channelopsin-1 and 2 (Chop1/2) by us (Nagel et al., 2002; 2003) and CSRA/B (Sineshchekov et al., 2002) or Acop1/2 (Suzuki et al., 2003) by others.

The hydrophobic core region of the proteins shows homology to the light-activated proton pump bacteriorhodopsin. ChR-2 (ChR2 = Chop-2 + retinal) is a cation channel,which is directly switched by light, as shown by expression in *Xenopus laevis* oocytes (Nagel et al., 2003). The action spectrum of ChR2 has its maximum at 460 nm (Sineshchekov et al., 2002; Nagel et al., 2003).

We show heterologous expression of ChR-2 in mammalian cells. Whole cell patch clamp studies demonstrate large light-gated ion currents and the capacity of ChR2 to depolarize the membrane by illumination. ChR-2 or truncated ChR-2 (ChR2-315), when expressed in mammalian cells, yields light-gated channel activity with no apparent difference from *Xenopus laevis*-expressed ChR2. A fusion protein of ChR2-315 with yellow fluorescent protein (eYFP) induced strong fluorescent labeling of the plasma membrane, indicating its preferential expression there. ChR2-315-eYFP showed the same light-induced activity as ChR2 itself. *[Supported by the German Research Foundation (DFG, in SFB 472) and the Max-Planck-Society.]*

P-893

Effect of pH on photosynthetyc redox-reactions around photosystem II of the thylakoids

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The efficiency and rate of photosynthetic water-oxidation activity and photosynthetic electron transfer reactions are dependence on pH. Dependence oxygen evolution (OE) with PpBQ and ferricianide, the amplitude of the multiline EPR-signal at 6°K associated with S_2-state and millisecond delayed light emission (ms-DLE) both for chloroplast thylakoid and oxygen evolving complex have shown that amplitude of OE from thylakoid and multiline EPR signal on pH in range of 4,0-9,0 decreased in alkaline and acetic pH and stable in range of 5,8-7,2. The loss of OE and S_2-state correlated with the loss of the slow component of DLE of chlorophyll in thylakoid membranes is observed. At pH 4,5 remained about 10 % of amplitude of the multiline EPR signal and OE and at pH 8,5 remained about 35-40 % of amplitude of the multiline EPR signal and 18 % OE observed at pH 6,5. The intensities of the ms-DLE have maximum value at pH 7,5 whereas maximum EPR signal amplitude is observed at pH 6,5. Both alkaline and acidic pH inhibits photoreactions of PSII, which leads to the loss of light-induced amplitude of multilane EPR signal, OE ability and ms-DLE of chlorophyll in thylakoids, showing that intrathylakoid pH plays essential role in photosynthesis functioning and regulation.

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Spectral and redox characterization of the novel heme ci in the cytochrome b6f complex

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A new *c*'-type heme was recently identified in the X-ray structure of the cytochrome b_6f complex. It is located in the quinone reducing pocket Q_i, inserted between the putative quinone binding region and heme b_i (presumably the high potential heme b_H). This was denoted heme c_i (Stroebel et al., 2003) or *x* (Kurisu et al., 2003). Previous *in vivo* spectroscopic data provided evidence for the existence, in thylakoid membranes, of such an electron carrier, then called "G", which can exchange electrons with b_H in a membrane potential-dependent manner (Lavergne, 1983; Joliot and Joliot). This has been the only information on the presence of this redox center until the structure was solved. In the present work, redox-induced absorbance changes were monitored in purified cytochrome b_6f complex from *Chlamydomonas reinhardtii*. Four spectral components with midpoint potentials at pH 7 of +340 mV (cyt *f*), -30 mV (cyt b_H), -130 mV (cyt b_L), and +100 mV were found. The latter component, ascribed to heme c_i, shows a broad absorption band around 427 nm and no significant change in the green region, in agreement with known spectra of *c*'-type cytochromes. These results are discussed in relation to structural and functional information on the cytochrome b_6f complex.

P-895

Role of LHCII organization in the process of freeze-thaw damage of thylakoid membranes

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The low temperature (77K) chlorophyll fluorescence, photochemical activity, oxygen flash yield and oxygen burst decay of thylakoid membranes with different organization of the light-harvesting chlorophyll a/b complex of photosysytem II (LHCII) were investigated after freeze-thaw cycle in criotoxic and cryoprotective medium. The increase of LHCII oligomerization, which is associate with significant reduction of the surface charge density of the thylakoid membrane, correlates with lower extent of freezing damage of the photosynthetic apparatus, when the procedure is carried out in cryotoxic medium (NaCl). In the presence of the cryoprotective compound (sucrose) freezing damage is less pronounced and is not affected by the degree of the LHCII oligomerization. The mechanisms of damage and protection of photosynthetic apparatus in the process of freeze-thaw treatment are discussed.

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– Light Driven Systems –

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Molecular architecture of the HAMP domain of NpHtrII investigated by SDSL EPR

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Sensory rhodopsin II from *N. pharaonis* (NpSRII) forms a complex with its cognate transducer NpHtrII in a 2:2 stoichiometry[1]. Light activation of NpSRII leads to a movement of helix F which triggers a rotation of TM2 in NpHtrII[2,3]. The mechanism of signal transduction through the HAMP region to the cytoplasmic domain of the transducer is still unknown. Structural information exists for the transmembrane and cytoplasmic regions, however the HAMP domain is not yet characterized. In order to obtain structural information on this domain, twenty-four residues in the membrane adjacent region (78-101), and six residues in the following region were spin labeled and investigated by cw and pulsed X-band EPR. To analyze the overall architecture of the complex, doubly spin labeled variants between the transducer and the receptor were also engineered. Furthermore, effects induced by the presence of detergents and salts gave new insights on the structural properties of the HAMP domain. The data are discussed with regard to the phototransduction of the signal.
(1) Gordeliy,V.I. et al. 2002, Nature 419, 484 (2) Wegener, A. A., Klare, J. P., Engelhard, M., Steinhoff, H.-J. 2001, EMBO J. 20:5312 (3) Klare,J.P., Bordignon,E., Engelhard,M., Steinhoff,H.-J. 2004, Photochem. Photobiol. Sci. 3, 543

P-897

Protochlorophyllide oxidoreductase takes an abnormal reaction pathway below the glass transition

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Motions through the energy landscape of proteins lead to biological function. At temperatures below a dynamical transition (150-250 K), the activity of some proteins cease. In this work, we describe an enzyme that, instead, engages into a non-productive pathway below 160K. Protochlorophyllide oxidoreductase (POR) catalyzes the reduction of protochlorophyllide (PChlide) into chlorophyllide (Chlide), a key step in chlorophyll biosynthesis. POR is one of the two enzymes known to require light for catalysis.
When illuminated with gentle light at ∼165 K, the complex of *T. elongatus* POR with Pchlide and NADPH transforms into a non-fluorescent intermediate. Upon warming, several fluorescent intermediates develop, and at ∼290K Chlide is released. When illuminated at temperatures below 155K, POR behaves differently. If gentle light is used, the reaction can not start. Instead, if a blue laser source is used, the initial complex disappears, like at 165K. However, upon warming, a new intermediate develops that fluoresces at 694nm and leads to a dead-end product.
By using fluorescence microspectrophotometry, we have measured the solvent glass transition temperature of the system to be ∼158K. The solvent glass transition, possibly controlling a POR dynamical transition, may be the determinant that switches the enzyme reaction pathway from a non productive to a productive one. The non-productive pathway results from a two-photons absorption mechanism, whereas the productive pathway is a one-photon mechanism.

P-898

Functional effects of a point mutation in the D2 protein that lowers the redox potential of Q_A

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We created two site-directed mutants, A249S and L267I in the D2 protein of Photosystem II in *Thermosynechococcus elongatus*. Both mutations are within the binding pocket of the primary quinone acceptor (Q_A). We investigated the effects of the mutations *in vivo* and in isolated PSII. While the L267I mutant exhibits characteristics similar to the wild type, the A249S mutation effects Q_A– charge recombination measured by thermoluminescence and fluorescence-decay. These results strongly indicate that the A249S mutation induce a shift in the redox potential of Q_A. The A249S accelerates the rate of photoinhibition, an effect consistent with the negative shift in the redox potential. EPR was used to measure the temperature dependence of the electron transfer from Q_A– to Q_B in the A249S mutant. It was found to be indistinguishable from the wild type despite the difference in the midpoint potential of Q_A. This is taken as an indication as a gating mechanism on the acceptor side of PSII similar to that in bacterial reaction centers.

P-899

Pigment composition and properties of the mutant reaction center of Rb. sphaeroides I(L177)H

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The photosynthetic reaction center (RC) of the purple bacterium Rb. sphaeroides is the membrane pigment - protein complex, where light – induced charge-separation occurs. The protein environment surrounding the cofactors of the primary electron transfer can affect the efficiency of this process. Using method of site-directed mutagenesis we obtained the mutant RC of Rb. sphaeroides with substitution of Ile in L-177 position by His. The mutant strain forms stable photochemically active RC complexes. Spectral and photochemical properties of the mutant RC differ significantly in the absorption bands corresponding to the primary donor and the monomer BChl absorption. It was shown, that the pigment content in the mutant RC I(L177)H has changed. The mutant RC contains only three BChl molecules comparing to four BChl molecules in the wild type RC. EPR linewidth of the photoinduced signal of the primary donor P870 was different in the wild type and I(L177)H mutant chromatophore preparations (1.05 mT and 1.3 mT at T=10 K, respectively). This is an indication of different spin delocalization in the primary donor, for the mutant being typical of a monomeric oxidized BChl. Considering the fact that the properties of both isolated and membrane-associated mutant RCs were similar, we conclude that missing BChl molecule from the mutant RC was the result of the introduced mutation but not of the protein purification procedure. Authors acknowledge the support by the Russian BRF.

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P-900

Phototactic responses of *O. flava* during its life cycle

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The life cycle of *Ophryoglena flava*, a histophagous ciliate dwelling in fresh waters, reportedly includes several stages that feature morphology changes and different phototactic responses. Previous studies on the phototactic responses in *O. flava* during its phase of maximal positive phototaxis led to an action spectrum with two main peaks at 420 and 590 nm, and a minor peak at 540 nm. Starting from those results, we analyzed the phototactic response at various cell ages, using three broad-band interferential filters (FWHM = 50 nm) centred respectively at 420, 550 and 600 nm, and constructed dose-effect curves for each band. A higher photosensitivity at 420 nm, and lower photosensitivies with the other two filters (550 and 600 nm) have been observed at any cell age. However, the photosensitivities in the blue and orange regions show a different time course vs. cell age with respect to the photosensitivity in the green region.
Measures were also carried out on cells whose feeding cycle was altered by a 4-day starvation (a double time with respect to the standard protocol) before being fed at t = 0. The maximal photoresponse values reached by starved cells are lower than the highest values reached with standard cultures; in other words, a general reduction of the phototactic response is observed. These results suggest that, while feeding optimally induces cell division, it does not generally reset all cellular functions.

P-901

Sudden polarisation and coherent vibration in bacteriorhodopsin

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Sudden polarisation – a large change in the electric dipole moment between the excited and the ground state – is a well-known phenomenon for retinal chromophore. Some early models of the energy transduction mechanism in bacteriorhodopsin (BR) even attribute a primary functional role of that. However, it was apparently unrecognized that the Maxwell theory intuitively predicts the appearance of an ultrafast transient electromagnetic radiation due to this dipole moment change. Here we show that the existence of this type of radiation can be derived from semiclassical quantum electrodynamics as a second order phenomenon. In optical terms it corresponds to the previously unstudied resonant case of optical rectification. Recently we experimentally observed a major component in the fs coherent infrared emission of oriented purple membranes of BR corresponding well to this effect (Groma et. al, *Proc. Natl. Acad. Sci.* 101, 7971, 2004). Our theory predicts that such a signal holds detailed information on the dynamics of excited state polarization, opening a new branch of impulsive spectroscopy on asymmetric systems. Beyond optical rectification we found a complex phase a coherent oscillation living for a few ps, i.e. much longer than the excited state of BR. Fitting analysis resulted in at least seven vibrating modes in the 700-1500 cm^{-1} region, while windowed Fourier transform indicated time-dependent frequency distribution.

P-902

Effect of UV-A radiation on thylakoid membranes with different organization

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The effect of UV-A (320-400 nm) radiation on the energy transfer and the photosynthetic oxygen evolution of thylakoid membranes from pea mutants was investigated. The membranes have different pigment composition, stoichiometry and organization of pigment-protein complexes. The aim of our work was to find out whether UV-A induced damage is affected by the altered content and/or oligomerization of the main light-harvesting chlorophyll-protein complex (LHCII) in thylakoid membranes.
The data for the effect of UV-A radiation on the oxygen evolution demonstrate that: (i) the inhibition of photosystem II (PSII)-mediated electron transport and flash-induced oxygen yields strongly depend on the amount of LHCII; (ii) the increase of the S_o populations of PSII centers in darkness is more pronounced in thylakoid membranes with smaller amount of LHCII; (iii) the inhibition of the oxygen evolution is related to the reduced number of the functionally active PSIIα centres; (iv) the degree of impairing of active PSIIα centres depend on the amount and oligomerization of LHCII. The results also show that the altered content and organization of LHCII influence the UV-A light-induced changes in the energy transfer between PSII and PSI and within the supramolecular LHCII-PSII complex. The effects of UV-A radiation on leaves and isolated thylakoid membranes are compared.

P-903

Electrostatic potential of archaeal rhodopsins: implication for their absorption spectra

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Depending on their function, the absorption spectra of rhodopsins can be tuned by the protein over a wide range. A major determinant for spectral shifts between different rhodopsins are electrostatic interactions between the chromophore retinal and the protein. We compute and compare the classical electrostatic potential at the retinal of three archaeal rhodopsins: bacteriorhodopsin (BR), halorhodopsin (HR), and sensory rhodopsin II (SRII). These proteins are an excellent test case for understanding the spectral tuning of retinal. The absorption maxima of BR and HR are very similar, while the spectrum of SRII is considerably blue shifted. We find that the electrostatic potential is similar in BR and HR, but differs significantly in SRII. A quantum mechanical model of a particle in a box with a step potential can qualitatively relate the differences between the electrostatic potentials of the proteins to the relative shifts of their absorption maxima. By decomposing the electrostatic potential into contributions of individual residues, we could identify six residues that are responsible for the differences in electrostatic potential between the proteins. Three of these residues are close to the retinal, while the other three residues are more then 8 Angstroem away from the retinal. The counterion of the Schiff base, which is frequently discussed to be involved in the spectral tuning, does not contribute to the dissimilarities between the electrostatic potentials.

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P-904

Role of LHCII organization in the process of freeze-thaw damage of thylakoid membranes

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The low temperature (77K) chlorophyll fluorescence, photochemical activity, oxygen flash yield and oxygen burst decay of thylakoid membranes with different organization of the light-harvesting chlorophyll a/b complex of photosystem II (LHCII) were investigated after freeze-thaw cycle in cryotoxic and cryoprotective medium. The increase of LHCII oligomerization, which is associate with significant reduction of the surface charge density of the thylakoid membrane, correlates with lower extent of freezing damage of the photosynthetic apparatus, when the procedure is carried out in a cryotoxic medium (NaCl). In the presence of a cryoprotective compound (sucrose) freezing damage is less pronounced and independent of the degree of the LHCII oligomerization. The mechanisms of damage and protection of photosynthetic apparatus during the freeze-thaw process are discussed.

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P-905

Microsecond time-resolved X-ray diffraction study of purple membrane

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The structural changes in the photoreaction cycle of bacteriorhodopsin, a light-driven proton pump, was investigated at a resolution of 7 Å by time-resolved X-ray diffraction experiment utilizing synchrotron X-rays from an undulator of SPring-8. The X-ray diffraction measurement system, used in coupling with a pulsed YAG laser, enabled to record diffraction pattern from purple membrane film at a time-resolution of 6 μsec over the time domain of 5 μsec to 500 msec. In the time domain, the functionally most important M-intermediate appears. A series of time-resolved X-ray diffraction data after photo-excitation showed clear intensity changes caused by the conformational changes of helix G in the M-intermediate. The population of the reaction intermediate was prominent observed at around 5 msec after a photo-stimulus. In contrast, absorption measurement indicated the deprotonation of the Schiff base predominantly occurred at around 300 μsec after photo-stimuli. These results showed that the conformational changes characterizing structurally the M-intermediate predominantly occur at a later stage of the deprotonation of the Schiff base. Thus, the M-intermediate can be divided into two metastable stages with different physical characteristics.

P-906

Finite element model to predict the electric potential distribution in PS I containing vesicles

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Photosynthetic reaction centers (RC) are integral membrane proteins and molecular photovoltaic structures. Recently, it was suggested their use as triggers of voltage-gated ion channels in excitable cells, where a certain voltage threshold has to be reached to evoke a response (Kuritz et al., *IEEE Trans. Nanobiosci.* in press 2005). Experimental studies with RC's reconstituted in lipid vesicles have shown different values of transmembrane voltage, depending on parameters like light intensity, RC concentration and membrane passive properties. Ultimately, the purpose of this work is to have a tool to estimate the proximity, number and density of RC's required near a voltage-gated channel to activate an excitable cell. As a starting point, we aim to predict the spatial distribution of the membrane potential in vesicles. A finite element model was realized using a commercial package (FEMLAB, Comsol A/S). The three-dimensional distribution of the electrical potential near a single RC in the surface of a spherical vesicle was calculated. In terms of density, in conditions of saturating light, a minimum of $1{,}8e^{12}$ RCs/cm^2 is needed to develop a potential of 20 mV, capable to activate voltage-gated sodium channels.

P-907

Bioinspired complexes for water oxidation: photoinduced electron transfer and radical formation

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Coordination complexes based on a photoactive Ruthenium-polypyridyl moiety linked to simple, rigid ligands with binding sites for transition metals, are developed to mimic the light induced charge separation and water oxidation processes taking place in the photosynthetic apparatus.
Inspired by the structure around the donor side of photosystem II a family of phenanthroline based ligands holding an imidazole, a phenol or an indole unit simulating the amino acids histidine, tyrosine and tryptophan in the oxygen evolving complex, were developed as models for proton-coupled electron transfer. In some of the molecules investigated the hydrogen bonding interaction present in the natural system is reproduced. Combined data from photophysical, spectroelectrochemical studies and DFT calculations evidenced the photogeneration of a phenoxyl or a tryptophan radical upon excitation of the chromophore in presence of an external electron acceptor, therefore mimicking the electron trade between $P_{680}{}^+$ and TyrZ-Hist190.

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P-908

Bidirectional electron transfer in Photosystem I from Chlamydomonas reinhardtii

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The recent crystal structure of photosystem I (PSI) from *Synechococcus elongatus* shows two quasi-symmetric branches of potential electron transfer cofactors including primary donor (dimer of chlorophylls P700), monomeric chlorophylls A and A_0 and quinone A_1, bound to the PsaA/PsaB heterodimer. So far, it is not clear if both potential electron transfer pathways are active in this process or only one of them. To solve this issue, we studied a set of 6 mutants with methionine coordinating the primary electron acceptor, A_0, replaced by histidine, leucine, or serine in either of two branches. Our results obtained with a technique of femtosecond transient absorption spectroscopy show that both branches are equally active in electron transfer. Mutation in either branch slows the forward electron transfer between A_0 and A_1 from $\sim$20 ps in wilde type PSI to 1-2 ns in all these mutants. This strong effect is explained by significant change in the redox midpoint potential and change in the position of A_0 by the mutations.

P-909

Phase diagram of two dimensional colloids: implications for membrane protein organization

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Recently high resolution images of bacterial photosynthetic membranes have revealed the organization of membrane proteins in these native membranes. The organization revealed is remarkable, and all the more so when we realize that these specialized, protein rich, membranes differentiate from the cytoplasmic membrane which has a more complex composition and is richer in lipids. Analysis of the protein organization in these specialized membranes from several different bacteria suggest that the organization results from a phase separation of several different contiguous phases.
In order to better understand our observations we have undertaken an examination of the different phase behaviors that are possible for membrane proteins considered as a two dimensional colloid. Monte-Carlo modeling of the phase diagram of this system shows the importance of interaction distance in the determination of system behavior. Transcription of our observations on the model systems to the photosynthetic membranes suggests that electrostatic and elastic forces in the membrane are of particular importance in determining the high level order of membrane proteins.

P-910

Structures of intermediates, functional relaxations and kinetics from time-resolved X-ray data

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Recent developments [Schmidt et al., 2003, Biophys. J. 84 2112; Schmidt et al., 2004, PNAS 101 4799] allow the determination of the structures of intermediates from time-resolved X-ray crystallographic data. In parallel, functional relaxations and chemical kinetic mechanisms can be investigated. Experiments on three proteins are reported. In the L29W mutant of Myoglobin initial protein relaxations extend into the ns time-regime although the heme itself has completely relaxed even at the fastest times [Schmidt et al., 2005, PNAS, submitted]. Early relaxations are interpreted in the light of existing investigations on protein dynamics [Parak, 2003, Rep. Prog. Phys. 66 103]. From 1 ns to 1.3 ms the CO is uniformly absent from the heme iron and accumulates at the proximal Xe1 site. Final relaxation occurs in concert with the CO rebinding. The detailed analysis allows the determination of the structure of a short lived species. An almost complete description of the photocycle of the Photoactive Yellow Protein becomes possible by using time-resolved crystallography on the wild-type and mutant E46Q [Ihee et al., 2005, PNAS, in press; Rajagopal et al., 2005, Structure 13, 55]. Relaxations were analyzed by singular value decomposition. 4 relaxation times, each, were determined. By fitting kinetic mechanisms the structures of photocycle intermediates were identified and refined. The SVD based analysis will pave the way to a general application of time-resolved macromolecular crystallography to other proteins and enzymes.

P-912

Cytokinin meta-topolin induced modification of photosynthetic apparatus in senescing wheat leaves

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We have studied the effect of a cytokinin *meta*-topolin (MT, 10^{-4} M) on senescence-induced changes in the photosynthetic apparatus of detached primary leaves of wheat (*Triticum aestivum* L. cv. Hereward). The senescing leaves were kept under continuous light conditions. MT significantly slowed down the senescence-induced decrease in chlorophyll content and markedly stimulated violaxanthin→zeaxanthin (Z) conversion. The high Z content was maintained even after an hour in darkness. MT treatment caused also the appearance of an emission band F699 peaking at 698-700 nm. This emission band is attributed to aggregates of light-harvesting chlorophyll a/b-binding proteins (LHC), the production of which is associated with a higher Z content. The presence of LHC aggregates in MT treated leaves was documented also by electron microscopy imagines. Besides the LHC aggregation, MT induced also a decrease in photosystem I content which was documented by electrophoresis and 77K-fluorescent spectra. Supported by grants FRVS 3190/2005 and MSM 6198959215.

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– Light Driven Systems –

P-913

Three-dimensional structure of major light-harvesting antenna of photosystem II from cucumber

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The major light-harvesting antenna complex of photosystem II (LHC-II), the most abundant integral membrane protein, functions in light capture, energy transfer/distribution and photoprotection. LHC-II from different species or conditions shows different spectral properties and variation in polypeptide and pigment components. This indicates some specific function-related alterations in the organization of LHC-II. Here we report a 2.66-Å crystal structure of cucumber homo-trimeric LHC-II, organized in a perfect virus-like icosahedral particle. The electron-density map shows the reasonable existence of a chlorophyll (Chl) *a/b* mixed binding site in the complex. The occurrence and locus of lactucaxanthin (Lac) was seen directly for the first time. Based on the credible structure information, a mechanism of the energy transfer, regulation and excess excited energy dissipation under high light condition was proposed.

P-914

Characterization of photosystem II by prompt and delayed chlorophyll a fluorescence

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An approach to the investigation of structural and functional properties of the Photosystem II supramolecular complex in native photosynthesizing objects based on the registration of delayed chlorophyll *a* fluorescence is developed. Using a disc phosphoroscope, we register simultaneously: i) changes of the intensity of millisecond delayed fluorescence (decayed in 0.35 – 5.5 ms time range) during the transition of the photosynthetic apparatus from dark to light-adapted state; ii) changes of the intensity of delayed fluorescence decaying in different subintervals of the investigated time range; iii) dark relaxation curves at different moments of the transition; iv) changes of the intensity of prompt chlorophyll *a* fluorescence. The analysis of these data allows the correlation of the delayed fluorescence characteristics to particular processes occurring in the Photosystem II complex – proton or electrical gradient accumulation, changes in the redox state of quinone acceptors, changes in the pigment-protein complexes caused by different stress factors, for example temperature.

Posters

– Imaging Organisms –

P-915

Quantification by optical imaging of gene electrotransfer in mouse muscle and knee

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Optical imaging was evaluated for monitoring and quantification of the mouse knee joint and tibial cranial muscle electrotransfer (ET) of a luciferase encoding plasmid. The substrate of luciferase (luciferin) was injected i.p or locally in the muscle or the knee joint. Luminescence resulting from the luciferase-luciferin reaction was measured with a cooled CCD camera. Luminescence of the knee joint and muscle were higher after local than after i.p injection of luciferin, but both measurements were highly correlated. Local injection procedure was adopted. A significant correlation was observed between measurements *in vivo* and *in vitro* on the same muscle. Reproducibility of individual luminescence measurements was also verified, and the luminescence levels were clearly dependant of the amount of plasmid injected. *In vivo* luciferase in the electrotransfered knee joint was detected for two weeks. Intramuscular electrotransfer of 0.3 or 3 μg of plasmid led to stable luciferase expression for 62 days, whereas injecting 30 μg plasmid resulted in a luminescence fall two weeks after electrotransfer. These decreases were, at least partly, related to the production of antibodies against luciferase. Thus, optical imaging was shown to be a relevant technique to quantify variations of luciferase activity *in vivo* in one given tissue. Furthermore, evaluating the effective amount of luciferase in tissues from *in vivo* luminescence levels requires calibration since it relies on conditions of the enzymatic reaction and light absorption.

P-916

Evaluation of tumor response of breast cancer patients by diffusion weighted MRI

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Diffusion weighted MR imaging (DWI) measures the diffusion of water molecules in tissues and is quantified by apparent diffusion coefficient (ADC). DWI can be used to differentiate tumors from normal tissue and also can be used to monitor the response of tumor to chemotherapy.

Thirteen healthy volunteers and twelve patients were recruited for the study. DW images were obtained prior to therapy (n=10) and after three cycles of therapy (n=3). The mean ADC value of tumors ($0.83 \times 10^{-3} \pm 0.05$ mm^2/s) was significantly less ($p < 0.05$) compared to the normal tissue ($1.80 \times 10^{-3} \pm 0.2$ mm^2/s). Decrease in ADC in tumor is due to an increase in the cellularity which restricts the diffusion of water molecules. In patients receiving neo-adjuvant chemotherapy, the ADC values were higher ($1.36 \times 10^{-3} \pm 0.86$ mm^2/s) and were closer to that of the normal tissue ($p < 0.05$), indicating response of the tumor to chemotherapy.

The post-therapy increase in ADC is due to the cell damage caused by the therapeutic agents which increases the fractional volume of the interstitial space, causing an increase in the mobility of water. The study showed that DWI can be used non-invasively to assess the response of breast cancer patients to neo-adjuvant chemotherapy.

P-917

Effects of 1.5 T magnetic field on T1-T2 relaxation time and trace elements

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The effects of static magnetic fields on humans have been the subject of continuous investigations. Since one of the major static magnetic field sources is nuclear magnetic resonance imaging (MRI), the present study aimed to investigate the effects of 1.5 T magnetic field that is produced by MRI on humans.

The study is carried out with 33 voluntary and healthy young men from 20 to 25 years of age. The subjects informed about the purpose of the study at the beginning. The subjects exposed to 30 minutes of 1.5 T static magnetic field by means of putting the subjects into the magnetic resonance unit. 5 ml blood was taken from each subject one minute before and one minute after exposure.

T1 and T2 relaxation times and trace elements were measured in of pre and post exposure plasma of the subjects. The obtained post exposure values were compared with pre-exposure values of the subjects. Pre and post exposure results were analyzed by means of Student t-test.

P-918

Refractive effects in Coherent Anti-stokes Raman Scattering (CARS) microscopy

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Coherent anti-Stokes Raman scattering microscopy (CARS) is a new approach for chemical imaging of molecular systems within cells and tissues, with high sensitivity, high spatial resolution, and three dimensional sectioning capabilities, without using fluorophores that are prone to photobleaching. This technique permits to map selectively molecular species, by using vibrational properties of their chemical bounds. The Epi detected (E-CARS) and forward detected (F-CARS) intensities depends on the shape, the size of the sample, as well as the index of the solvent. In this presentation, after introducing the CARS microscopy technique, we show the first CARS studies of the refractive effect of the sample, comparing the E-CARS and F-CARS signals for different diameters of polystyrene beads, in different refractive index solvents. We present several simulations, comparing forward-detected and backward-detected signals in different sized polystyrene beads, embedded in different index solvents, and we show that, the backward-reflected F-CARS dominates the experimentally epi-detected signals. Furthermore, we demonstrate experimentally and theoretically that the maxima of forward and epi-detected signals are generated at different positions along the Z axis in the sample. We finally discuss how index mismatch in cells can alter CARS images.

Posters

– Imaging Organisms –

P-919

Study about secondary effects of non specific contrast media in MRI

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Introduction: A study on human patients has been made to compare the tolerance of gadolinium based contrast media with data from manufacturer and other published data.
Materials and methods: We report a study period from 1998 till 2004 with a number of 3986 patients involved in this study. For Human investigation a MRI scanner Siemens Magnetom was used. Gadolinium based contrast media from different manufacturers was intravenous injected to obtain a better image. Any side effects, if appeared, were recorded and statistically evaluated.
Results and Discussion: The total amount of patients with side effects after gadolinium contrast media administration as shown is 0,075 %.This is a number a little bit higher than other literature is mentioning, in other bigger studies (over 20000 patients) the result was 0,0003%, but in bought results we can observe that gadolinium contrast media is very good tolerated by human patients.
Conclusions: Gadolinium based macrocyclic complexes produced as contrast media for human MRI examinations have good paramagnetic efficacy, good stability and selectivity in respect of the gadolinium ion, are biologically very good tolerated and produced results with a high clinical relevance.

P-920

Cell-silicon synapse: vesicle exocytosis monitored by field-effect transistors

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We cultured bovine chromaffin cells on an array of electrolyte-oxide-silicon field-effect transistors (EOS FET) and monitored granule secretion. By stimulation with barium chloride, vesicles are released into the narrow sheet of electrolyte between the chip surface and the plasma membrane. The interaction of released protons with the silicon dioxide surface of the chip alters the threshold voltage of the transistor and gives rise to a measurable signal. Simultaneously performed measurements with a carbon fibre showed a correlation of the transistor signals and amperometric current traces. We conclude that the transistors are able to monitor exocytosis on a single vesicle level.
To elucidate the role of protons, we destroyed the proton gradient across the vesicle membrane by nigericin and valinomycin. As a result a massive reduction of the transistor signals was induced, whereas there was only little change of the amperometric records. We conclude that released protons are responsible for the detection of vesicles with transistors.
The individual transistor records of vesicle exocytosis can be explained by combining the dynamics of the exocytotic event with the diffusion in the cell-chip junction.
Transistor recording of exocytosis does not depend on the electrochemistry of transmitters. As many kinds of exocytotic vesicles contain a large amount of buffered protons it can be applied to numerous kinds of exocytotic events, independent on the nature of the transmitter.

P-921

In vivo subcellular structures recognized with phase

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Phase contrast transmission electron microscopy has been developed to enable a high contrast and a high resolution observation for unstained ice-embedded samples. To enhance the image contrast, two methodologies have already been developed; i) scattering contrast for stained samples with small aperture diaphrams and ii) defocus contrast for unstained or stained samples with deep defocusing. The former prevails in histochemical sciences and the latter is popular in electron crystallography. Both methods, however, have a common drawback that the contrast is only improved by impairing the image quality. This drawback can be removed with use of the phase contrast method using phase plates, which has traditionally been used in visible light microscopy. Due to the severe obstacle of the charging of phase plates, however, the idea has not yet been materialized. We have solved the phase-plate charging problem. An experiment 300kV with TEM for a whole cell from cyanobacterium unstained and ice-embed fulfilled the expectation. Only weak and vague contrast was obtained for the conventional image of the cell even with a very deep defocus. Contralily a high-contrasted image has appeared for phase contrast images, where various fine structures are clearly recognized. This may be a first example to observe nanometer scale structures in details in the intact cell. Other examples including intact state intravesicular structures will be shown.

P-922

Acute effect of corticosterone on NMDA receptor-mediated Ca2+ elevation in mouse hippocampal slices

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Corticosterone (CORT) is a principal glucocorticoid synthesized in the rodent adrenal cortex and secreted in response to stress. We examined the rapid effects of CORT on *N*-methyl-D-aspartate (NMDA) receptor-mediated Ca^{2+} signals in adult mouse hippocampal slices by using Ca^{2+} imaging technique. Application of NMDA caused a transient elevation of intracellular Ca^{2+} concentration followed by a decay to a plateau within 150 sec. The 30 min preincubation of CORT induced a significant decrease of the peak amplitude of NMDA-induced Ca^{2+} elevation in the CA1 region. The rapid effect of CORT was induced at a stress-induced level (0.4-10 μM). Because the membrane non-permeable bovine serum albumin-conjugated CORT also induced a similar rapid effect, the rapid effect of CORT might be induced via putative surface CORT receptors. In contrast, CORT induced no significant effects on NMDA-induced Ca^{2+} elevation in the dentate gyrus. In the CA3 region, CORT effects were not evaluated, because the marked elevation of NMDA-induced Ca^{2+} signals was not observed there.

Posters

– Imaging Organisms –

P-923

Personal histories of exocytosing vesicles revealed by 3D TIRFM ´retrotracking´ in BON cells

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We have used total internal reflection fluorescence microscopy (TIRFM) to visualizedynamics of NPY-GFP labelled secretory vesicles near the basal plasma membrane ofBON and chromaffin cells which are specialized in secreting serotonin and adrenalin, respectively. Stimulation of secretion via entry of extracellular Ca2+through digitonin-induced pores caused some, but not all vesicles near the basal cell membrane, to exocytose. In orderto understand factors that distinguished vesicles which exocytosed from those which didnot, we looked at the individual histories of exocytosing vesicles (EV) by 3D tracking. Approach of a subset of EV toward the cell membrane prior to fusion informed us on time and length scales involved in becoming release-ready.

P-924

Confocal microscopy of the phototactic ciliate *F. salina*.

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Fabrea salina is a marine heterotrich ciliate, which dwells in salt ponds. In previous works we have described the phototaxis and the fluorescence properties of a hypericin like endogenous pigment in an albino strain. We have recently obtained a heavily pigmented strain from the saline of Torre Colimena (Taranto, Italy). We have used confocal microscopy to characterize the fluorescent properties of this strain and to compare them with those of the albino strain. The results obtained by one and two photon confocal microscopy show that, as in the albino strain, the fluorescence intensity of the pigment is higher in dead cells than in the living cells. The excitation and emission spectra are quite similar in the two strains and this is also true for the fluorescence lifetime, which is about 2 ns. All together, these measurements indicate that the pigment of the new strain belongs to the family of hypericin-like chromophores. The analysis of different confocal planes shows that the pigment is localized not only in granules under the somatic membrane in the cellular body, as currently thought, but also in the cilia. Some experiments of fotobleaching "in situ" confirm this result, that might have important implications in the understanding of the mechanisms of the photomotile responses of *F. salina* and probably of other heterotrich ciliates.

P-925

Insoluble fluorescent particles and biocompatibility

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Autoxidation of an aminophospholipid dispersed in non-polar solvents produces fluorescent particles insoluble in water and most organic solvents. The fluorescence emission maxima was in the region of 430-440 nm and excitation maxima in the region of 360-370 nm. There was no significant difference in excitation maximal with reaction time, indicates the stability of fluorophores in the product. We tried various solvents for the solubility of the fluorescent product, and found that the product was insoluble in water and most organic solvents. A quite bright fluorescence emitted by the particles was observed by fluorescent microscope when emitted by UV365 nm. SEM indicated that the size of the particles was 1μm$\sim$20μm, depending on the reaction time and phospholipid concentration in hexane solution. Endothelial cells from human vein grew better on the surface prepared from the particles than the culture plate, implies a possible application as a new type of biomaterial as a coating material for medical devices, and as a fluorescent tracer for human bodies.

Posters

– Teaching Biophysics –

P-926

Dynamics of science issues in a basic biophysics course for biochemistry students

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Learning on science is also learning how scientific knowledge is produced. In this sense, issues related to the dynamics of science should be brought into focus in science education. The idea has support in the 1999 "Declaration on Science and the Use of Scientific Knowledge", particularly in the statement that "science curricula should include science ethics, as well as training in the history and philosophy of science and its cultural impact".
Thinking on first year undergraduate students, we are interested in an integrated approach of that kind of themes. This presentation describes a practical teaching module included in the basic biophysics course for biochemistry majors. Organized in case studies, it deals with stories of biophysics (and biochemistry), and addresses the role of the biophysical approach in the progress of the life sciences. The module follows the whole course, and consists in small exercises on the way a given understanding has been constructed explored within the practice trend of contemporary science studies. Examples of the chosen stories – anchored in the subjects covered in the lectures of the course – relate to the search for the mechanism of energy production through ATP-synthase, the development of radioactive labelling techniques, and the discovery of protein water channels. Beyond their value as cultural legacy and as motivating tools, the insight they might provide is vast.

P-927

Teaching biophysics in medical school

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There is no doubt that modern physician should have the knowledge of basic sciences as Physics, Chemistry and Biology. Furthermore, the Biophysics is incorporated into the curriculum of most European Medical Schools. At Medical School in Zagreb, the course of Physics and Biophysics is positioned in first and forth year of study. The students learn basic physics phenomena of structure of matter, mechanics, thermodynamics, electromagnetism, optics and acoustics applied on the human body. Additionally, the interactions of the body with the surrounding are thoroughly discussed as the basis for different diagnostic methods. The arguing at our School is still going on where to include the content of this course. Should it be the autonomous course or the part of physiology and radiology courses in the problem based learning approach?! So far at Zagreb Medical School the biophysical courses are autonomous structured according to the Biophysics programs at other European universities. The highlighting is on seminar work and lab, encouraging the students for individual learning. The seminars are made more vivid and instructive for students by inclusion of different model devices constructed in our department

P-928

Biological Physics in Physical Review E and Physical Review Letters

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Topics in soft matter and biological physics are published in both Physical Review E and Physical Review Letters. Both journals are indexed in Medline. In 2004, 3778 manuscripts were submitted to PRE and 9976 were submitted to PRL. Manuscripts are generally sent to two referees initially for review. Approximately 70% of the review requests result in reports. The median time for the first referee report is approximately 30 days. For direct submissions to PRE, the median time from submittal to acceptance is about 111 days for regular articles and Brief Reports and 97 days for Rapid Communications. For letters published in PRL in 2004, the median time from submittal to acceptance was 140 days. The acceptance rate is approximately 63% for PRE and 34% for PRL. The 2003 impact factor is 2.2 for PRE and 7.0 for PRL. Authors and referees come from the international community. In 2004, 79% of the manuscripts submitted to PRE had corresponding authors outside the US, and PRE used 4635 referees from outside the US and 3615 from inside the US. We welcome your feedback about the journals and the review process.

P-929

Biophysics in context of Agenda 21

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Introduction: Agenda 21 of UNO supports *better situation* of humanity via education, health, ecology, economy, etc.
Method: Statistical evaluation of numbers of *contributions* from countries in *scientific congr* related to *biophysics* is given, esp of IUPAB **B**uenos **A**ires 2002 (n=530), **N**ew **D**elhi 1999 (n=62), also *physiology* **Nice** 2003 (n=590), *pathophys.* **Bud**apest 2002 (n=628) and *rad res* **Bris**bane 2003 (n=943)**.**
Results: **(BA/ND/Nice/Bud/Bris) West Europe** *128/21/406/155/183* (France 27/7/158/9/15, Germany 27/6/36/43/42, GB 16/8/41/21/52), **East E** *59/1/95/116/29* (Russia 6/1/13/41/8), **China** *10/1/6/16/15*, **India** *15/10/0/3/25*, **Japan** *27/5/6/42/203*, **USA** 82/14/13/94/274.
Conclusion: Large discrepancies in presentations of (developing, industrial) countries are evident. *ICSD/Int Acad Sci* supports projects of *Int University* (IU: fac anthrop, informatics, med, etc) based on a *network* of national inst with *common* research, educ progr, personnel, whole life work, etc. This is conform with ideas of *I Kant* for foundation of exp. schools and *B Russel* - for IU helping for peace. Foundation of *first int inst for biophysics* could help essentially for Agenda. Info: ICSD, Postfach (POB) 340316, 80100 Muenchen, Germany.
Neu et al: Faseb J **19**/4 A1355 2005; Fund Clin Pharm **18**/S1 119 2004; J Biosci **24**/S2 3 1999

Posters

– Teaching Biophysics –

P-930

Axiomatic theory of biophysics

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Until now, all approaches to interpret biology by applying principles of physics have been announced failure. The achievement of biophysics is limited in area to provide essential tools for biological research and biophysics is far from to be the basic theory of biology. To resolve it requires the fundamental research about the logic features of biophysical processes of biology. We promoted system logic and then protein thermodynamics structure theory. Based upon it, the axiomatic theory of biophysics and biology could be developed. Our result shows that the real understanding of biology or biophysics must be constructed based upon new thinking methods.

Posters
– Drug Design and Delivery –

P-931

Inhibition of HIV Reverse Transcriptase activation by peptides and analogs

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Reverse Transcriptase (RT) plays an essential role in the replication of HIV and constitutes the main target for the development of AIDS therapies. The biologically active form of HIV RT is a heterodimer of two subunits, p51 and p66, each consisting of distinct subdomains: the fingers, the palm, the thumb, the connection and the RNase H subdomain, the latter only present in p66. We have demonstrated that formation of fully active RT is a two-step process involving rapid association of the two subunits (dimerization) followed by a conformational change (maturation). Thanks to the crystal structure of RT we have identified a new class of inhibitors based on short peptide motifs derived from the dimer interface. We first identified a short 9mer peptide (Pep-7) derived from the tryptophan-rich motif of the connection subdomain that blocks dimerization of RT and efficiently abolishes HIV-1 replication. Pep-7 interacts preferentially in a pocket involving residues Trp^{24} and Phe^{61} on p51. We then designed 15mer peptides derived from the thumb domain which inhibit RT maturation as well as viral replication when delivered into cells. Taking into account these results we propose that dimerization of RT constitutes a potential target for the design of more specific new antiviral drugs.
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P-932

Molecular modeling to circumvent cancer drug resistance associated with ABCG2

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Irinotecan is a widely-used antitumor drug that inhibits mammalian DNA topoisomerase I. However, overexpression of ABCG2 can confer cancer cells resistance to SN-38, that is, the active form of CPT-11. In the present study to develop a platform for the molecular modeling to circumvent drug resistance associated with ABCG2, we have characterized a total of fourteen new SN-38 analogues by some typical properties, which were evaluated by molecular orbital (MO) calculations and neural network (NN) analysis. The NN was first applied to estimate hydrophobic properties (LogP) of the analogues. Thereafter, the electrostatic potential (ESP) and the solvation free energy (ΔG) were evaluated by MO calculation. These indexes were found to be well correlated with the drug resistance ratio experimentally observed in ABCG2-overexpressing cells. It is suggested that hydrophilic analogues carrying OH- or NH_2-groups are good substrates for ABCG2 and therefore exported from cancer cells. In contrast, SN-38 analogues with Cl or Br atom at those positions have similar LogP values and high affinities toward the putative active site of ABCG2, however they were not substrates of ABCG2. From these results, it is strongly suggested that hydrogen bond formation with OH- or NH_2-groups are critically involved in the transport mechanism of ABCG2.

P-933

Antitumor agents into lipid multilayers

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Recently it was shown that liposomes made by cationic lipids have high affinity to angiogenic blood vessels around a solid tumor making them useful for targeted delivery of a drug to the tumor vasculature [1]. By neo-vascular targeting of paclitaxel-loaded cationic liposomes an improved retardation of tumor growth with respect to the conventional paclitaxel formulation was achieved [2].
We have studied the incorporation of the hydrophobic anti-cancer agent Paclitaxel (PXL) into a variety of lipid matrices by X-Ray and neutron difraction measurements. Liposome suspensions from cationic and zwitterionic lipids, comprising different molar fractions of Paclitaxel, were deposited on planar glass substrates. After drying at controlled humidity, well-ordered, oriented multilayer stacks were obtained, as proven by the presence of bilayer Bragg peaks to several orders in the reflectivity curves.
The presence of the drug induced a decrease of the lipid bilayer spacing, and with an excess of drug, also Bragg peaks of drug crystals could be observed. From the results, insight into the solubility of Paclitaxel in the model membranes and an electron density profile were obtained.
[1] Thurston G. et al. *J Clin Invest* **1998**, 101. [2] Schmitt-Sody M. et al. Clin Cancer Res **2003**, 9.

P-934

Cross-docking of highly flexible ligands using a multisolution-gsa docking method

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A successful ligand-receptor docking methodology depends strongly in the efficiency of the global optimization algorithm used to explore the ligand conformational space. In this work we have implemented and analyzed the performance of a new flexible ligand-receptor docking methodology. This methodology uses as optimization method a multisolution version of the generalized simulated annealing algorithm adapted to problems with box constraints. A grid-based methodology, considering the receptor rigid, and the GROMOS96 classical force field are used to evaluate the ligand-receptor scoring function. The methodology was tested in redocking (ligand within it own protein conformation) and cross-docking (ligand within another protein conformation) experiments for five HIV1 protease-ligand complexes with known three-dimensional structures. All ligands tested are highly flexible, having 12 to 20 conformational degrees of freedom. The implemented docking methodology was able to redock successfully all flexible ligands with a success ratio $\geq$ 95% and a mean RMSD lower than 1.52 Å with respect to the corresponding experimental structures. In the cross-docking experiments we observed a strong dependence of the mean success ratio with respect to the protein structure used as reference. In 4 situations we observed a mean success ratio $\leq$ 40% and $\geq$ 70% in 13 cases among the 20 possible ones.
Financial support by CNPq and FAPERJ: grants no. 402003/3003-9, E26/171.401/01 and E-26/170.648/2004.

Posters
– Drug Design and Delivery –

P-935

Mechanisms of non-covalent peptide mediated cellular delivery of therapeutics: a biophysical study

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Two different cell-penetrating peptides MPG and Pep-1 were shown to promote non-endosomal intracellular delivery of non-covalent bound cargos, namely nucleic acids and proteins; respectively. In order to identify the peptide mediated internalization pathway, we undertook conformational investigations of both peptides with and without associated cargos and checked the conformational consequences of the presence of phospholipids. From the conformational point of view, Pep-1 behaves differently from MPG. CD analysis revealed a transition from a non-structured to a helical conformation upon increase of the concentration while MPG remained non-structured. Determination of the structure by NMR showed that in water, it's a-helical domain extends from residue 4 to 14. CD and FTIR indicated that Pep-1 adopts a helical conformation in the presence of phospholipids while MPG is in a β-sheet form. Adsorption measurements performed at the air-water interface were consistent with the helical form. Pep-1 did not undergo conformational changes upon formation of a particle with a cargo peptide. In contrast, we observed a partial conformational transition when the complex encountered phospholipids. For MPG, interactions with nucleic acids generated a partial folding into β-sheet which was more pronounced in the presence of lipids. Electrophysiological measurements showed that both peptides, whether associated or not with their cargo, can induce transmembrane pore-like structures.

P-936

Engineering self-assembly peptides for targeted delivery of therapeutics and imaging agents

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Peptide-mediated delivery systems have recently emerged as a means to substitute or augment conventional drug and gene delivery technologies. These approaches are versatile and easily designed to incorporate a number of specific attributes required for efficient delivery of therapeutic and imaging agents. In particular, self-associating peptide domains can be utilized to construct stable and structurally well-defined protein-like assemblies displaying a series of cell-routing functions. More specifically, a peptide-based self-assembling intercellular delivery vehicle was designed by incorporating the 30-residue long tetramerization domain of the human tumor suppressor protein p53 (hp53tet). The resulting peptide tetramer displays 8 termini within its structure that allows for the simultaneous presentation of distinct cell targeting signal or functional domains. The fusion of polycationic sequences to the hp53tet domain promotes the cellular import of the resulting constructs into eukaryotic cells. This internalization event was dramatically enhanced for such multivalent peptides in relation to their monomeric counterparts. Peptides containing a nuclear localization sequence along with a polycationic sequence were found to shuttle reporter plasmids efficiently to the nucleus of cells. These results have important implications in the design and construction of novel targeted delivery vehicles.

P-937

Biophysical and biological examination of DNA/lipids complexes particles of virus-like structure designed for *in vivo* gene transfer

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The structure of complexes made from DNA and suitable lipids (lipoplexes Lx) was examined by Cryo Electron Microscopy. We observed a distinct concentric ring-like pattern with striated shells when using plasmid DNA. These spherical multilamellar particles have a mean diameter of 254 nm with repetitive spacing of 7.5 nm with striation of 5.3 nm width. Small angle X-ray scattering (SAXS) confirmed CryoEM data and revealed repetitive ordering of 6.9 nm, suggesting a lamellar structure containing at least a dozen layers. This concentric and lamellar structure with different packing regimes was also observed by cryoEM with linear dsDNA, ssDNA and oligodeoxynucleotides. For the first time, DNA chains could be visualized in DNA/lipid complexes. Such specific supramolecular organization is the result of thermodynamic forces, which cause compaction to occur through concentric winding of DNA in a liquid crystalline phase. CryoEM of T4 phage DNA packed either in T4 capsides or in lipidic particles showed similar patterns. SAXS suggested an hexagonal phase in Lx-T4 DNA. Thus, both lamellar and hexagonal phases may coexist in the same Lx preparation or particle and transition between both phases may depend upon equilibrium influenced by type and length of the DNA used. Organization of such nucleotidic supramolecular assemblies is relevant for prebiotic chemistry.

P-938

Characterisation of the physicochemical properties of novel hydrophobically modified low molecular weight chitosan derivatives and their complexes with DNA

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The success of gene therapy largely relies on the availability of vectors that would deliver the genetic material efficiently to the target cells with a minimal toxicity. In this context, our purpose was to evaluate as possible vectors a series of newly synthesized low molecular weight (5 kDa) chitosan derivatives grafted with dodecenoyl (DDC) groups at different percentages (3, 9, 16 and 25 %). In the absence of DNA, the Critical Micellar Concentration (CMC) of these derivatives in 20 mM MES buffer pH 6.5 was found to be strongly dependent on the percentage of DDC but not on pH or salt concentrations. This indicates that the DDC groups confer to the chitosan derivatives the potency to self-assemble probably in micellar structures: a property that may dictate the formation and the structure of their complexes with DNA. Next, we investigated by quasielastic light scattering the size and the surface charge of complexes of plasmid DNA with these derivatives at different pH, salt concentrations and N/P ratios (expressed in charged units of chitosan amines to DNA phosphates). We found the smallest and more positively charged complexes were obtained at pH 5.8 and N/P=5 in the absence of salt: a condition where the chitosan derivatives were fully protonated and in excess over the DNA phosphate groups.

Posters
– Drug Design and Delivery –

P-939

High throughput in-silico screening against flexible protein receptors

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Virtual screening of chemical databases to targets of known three-dimensional structure is developing into an increasingly reliable method for finding new lead candidates in drug development.
Based on the stochastic tunneling method (STUN) we have developed FlexScreen, a novel strategy for high-throughput in-silico screening of large ligand databases. Each ligand of the database is docked against the receptor using an all-atom representation of both ligand and receptor.
In the docking process both ligand and receptor can change their conformation. The ligands with the best evaluated affinity are selected as lead candidates for drug development.
Using the thymidine kinase inhibitors as a prototypical example we documented the shortcomings of rigid receptor screens in a realistic system.
We demonstrate a gain in both overall binding energy and overall rank of the known substrates when two screens with a rigid and flexible (up to 15 sidechain dihedral angles) receptor are compared. We note that the STUN suffers only a comparatively small loss of efficiency when an increasing number of receptor degrees of freedom is considered.
FlexScreen thus offers a viable compromise between docking flexibility and computational efficiency to perform fully automated database screens on hundreds of thousands of ligands.

P-940

Characterization of mixtures of DNA and nonionic polymeric agents for gene delivery in muscle

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A strategy to cure muscle disease is to introduce genes (DNA) into the muscle cell to correct or to add genes. Nonionic polymeric agents have emerged as an efficient vector to deliver DNA in the muscle. These polymers protect DNA from extracellular nuclease degradation by allowing the DNA diffusion throughout the muscle tissue. There is at present no understanding about how nonionic polymers enhance transfection in the muscle. The kind of interactions between these nonionic agents and DNA, DNA-nonionic polymeric agent mixtures and cell membrane are currently unknown. Also the structure of DNA-nonionic polymeric agent mixtures is not yet well defined. More information is needed to improve this delivery system. Neutron scattering (contrast variation) and light scattering were used to investigate the interaction between: DNA and nonionic polymers (PVP, di- and triblock copolymers). Furthermore, electrical measurements with the same polymer complexes and black lipid membrane were also performed. Depending on the polymer type there is either direct interaction with DNA or in other cases polymers exhibit strong interaction with the lipid membrane. An explanation for transfection efficiency of these nonionic agents in gene delivery to muscle will be given.

P-941

Targeted delivery of photosensitizers into the cancer cell nuclei enhances their cytotoxic efficacy

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The search for new pharmaceuticals has raised interest in locally-acting drugs which act over short distances within the cell, and for which different cell compartments have different sensitivities, *e.g.* photosensitizers used in anticancer therapy should be transported to the most sensitive subcellular compartments where their action is most pronounced. Earlier we have produced a number of modular recombinant transporters for locally-acting drugs comprising several functional modules for cell-specific targeting, internalization, escape from intracellular acidic vesicle, and targeting to the nuclei of melanoma cells overexpressing melanocortin receptors. Here we describe new transporters on the basis of epidermal growth factor which are specific for a wide variety of cancers. These transporters possess all necessary functional activities and deliver photosensitizers into the nuclei of human carcinoma cells to result in photocytotoxic effects almost 3 orders of magnitude greater than those of nonmodified photosensitizers.

P-941-B

Self-assembly of hydrolysed alpha-lactalbumin into nanotubes

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Nanotubes are formed by self-assembly of partially hydrolysed α-lactalbumin, a 14 kDa milk protein. There are several promising applications of these α-lactalbumin tubes, in food, pharmacy and nanotechnology. We studied the mechanism of self-assembly, the structure and the properties of the nanotubes. Limited proteolysis of the α-lactalbumin (by a serine protease) makes the molecule prone to self-assembly. In the presence of Ca^{2+} tubular structures are formed. Other divalent ions like Mn^{2+} and Zn^{2+} can also induce tubular self-assembly, while Mg^{2+} leads to random aggregation. Light scattering showed that the self-assembly is reversible, which is of relevance for controlled release applications. On the other hand, we could also make stable tubes by cross linking, which would be a requisite for several other applications. From AFM and SAXS measurements, we obtained values for the outer diameter: 21 nm; and the inner diameter: 8 nm. AFM and cryo-EM revealed the helical structure of the tube wall; it is a right-handed helix. By performing nano indentations with AFM we determined mechanical properties of the tubes. The tubes were shown to be relatively resilient upon small deformations; the elastic modulus is of the order of 0.1 GPa.

Posters
– Drug Design and Delivery –

P-942

Peptide-based vectors for the delivery of siRNA and PNA *in cellulo* and *in vivo*

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The dramatic acceleration in the identification of new nucleic acid-based therapeutic molecules has provided new perspectives in pharmaceutical research. However, the development of nucleic acid- and peptide-based therapeutics is limited by their poor cellular uptake and trafficking. With the aim of addressing these issues, we have designed a family of short amphipatic peptides for delivery of nucleic acids (MPG) and of peptide/PNA (PEP). These carriers consist of a hydrophobic moiety and a NLS-derived hydrophilic domain. They form stable non-covalent complexes with peptides, proteins, siRNA or PNA without any requirement for prior covalent cross-linking. Both MPG and PEP carriers enter cells rapidly, in a process involving membrane disorganization, independently of the endosomal pathway. MPG efficiently delivers short ODNs and siRNA into a wide variety of mammalian cell lines, without interfering with their biological function. PEP significantly improves delivery of PNA and peptides. Both carriers were used for the delivery of siRNA or antisense PNA targeting the cell cycle regulatory protein cyclin B1 in an animal model and were found to block tumor growth upon intravenous injection. We believe that MPG and PEP-based technologies will contribute significantly to the development of basic and therapeutic applications.

P-943

Uptake of β-galactosidase mediated by pep-1 is driven by the membrane potential *in vitro* and *in vivo*

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The cell-penetrating peptide (CPP) pep-1 is capable of introducing large proteins into different cell lines, maintaining their biological activity. Two mechanisms have been proposed to explain the entrance of other CPPs in cells, endosomal-dependent and independent. We evaluated the molecular mechanisms of pep-1-mediated cellular uptake of β-galactosidase (β-Gal) from *E. Coli*, in large unilamellar vesicles (LUV) and HeLa cells. Fluorescence spectroscopy and immunofluorescence microscopy were used to study the translocation. Internalization of β-Gal into LUV and protein functionality in HeLa cells were detected by enzymatic activity. β-Gal translocated into LUV in a transmembrane potential-dependent manner. Likewise, β-Gal incorporation was extensively decreased in depolarized cells. Furthermore, β-Gal uptake efficiency and kinetics were temperature-independent and β-Gal did not co-localize with endosomes, lysosomes or caveosomes. Therefore, β-Gal translocation was not associated with the endosomal pathway moreover transmembrane pores were not detected. These results indicated that the protein uptake *in vitro* and *in vivo* was mainly, if not solely, dependent on a physical mechanism governed by electrostatic interactions between pep-1 (positively-charged) and membranes (negatively-charged).

P-944

Treatment of bladder carcinomas using *in vivo* electroprorative delivery of recombinant BCG DNA vaccines and interleukin-23 DNA vaccine

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Intravesical immunotherapy with live bacillus Calmette–Guérin (BCG) is the treatment of choice for superficial bladder cancers. Nevertheless, a significant proportion of patients do not respond to this therapy, and adverse effects are common. Here we report the delivery and expression of recombinant mycobacterial DNA vaccines *in vivo* and demonstrate the ability of multicomponent DNA vaccines to enhance Th1-polarized immune responses. Splenocytes from immunized groups of mice were re-stimulated *in vitro* and examined for cytotoxicity against bladder tumour cells. We used four combined recombinant BCG DNA vaccines (*multi-rBCG*) for electroporative immunotherapy *in vivo,* and found that tumour growth was significantly inhibited and mouse survival was prolonged. Increased immune cell infiltration and induction of apoptosis were noted after treatment with *multi-rBCG* alone, with the interleukin-23 (*IL-23*) vaccine alone, and—most significantly—with their combinations. Thus, electroporation immunogene therapy using *multi-rBCG* plus *IL-23* may be an attractive regimen for the treatment of bladder cancer. This approach presents new possibilities for the treatment of bladder cancer using recombinant BCG DNA vaccines and *IL-23* DNA vaccine.

P-945

Maturation and inhibitor design of SARS-CoV 3CL protease based on a product-bound crystal structure

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Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel human coronavirus. Here we report that the $3CL^{pro}$ containing N- and/or C-terminal additional in-frame sequences underwent autoactivation to cleave the tags and yielded the mature protease *in vitro*. The 3-D structure of the C145A mutant protease shows that the active site of one protomer of the dimeric protease is bound with the C-terminal six amino acids of the protomer in another asymmetric unit, suggesting a possible mechanism for maturation. The crystal structure of this product-bound form shows that the active site has a P1 pocket that binds the Gln side chain specifically. In addition, the P2 and P4 sites are clustered together to accommodate large hydrophobic side chains. The tagged C145A mutant protein served as a substrate for the wild-type protease and the N-terminus was first digested (55-fold faster) followed by the C-terminal cleavage as shown by the SDS-PAGE analysis. The analysis of t analytical ultracentrifuge experiments reveals the remarkably tighter dimer formation for the mature enzyme (K_d = 0.35 nM) than for the mutant (C145A) containing the N-terminal (K_d = 17.2 nM) or the C-terminal 10 extra amino acids (K_d = 5.6 nM). Taken together, the study here provides insights to the design of our new structure-based inhibitors.

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P-946

Biophysical study of non-lethal stress response of cultured DC3F cells

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Stress factors may induce two kinds of responses in living cells: either cell death or adapting mechanisms. Our aim was to search for non-lethal effects of various stress conditions on cultured hamster lung fibroblasts (DC3F cells) as well as to assess the recovery time after stress removal. DC3F cells were cultured in standard conditions and were submitted to stress, either by incubation with chemical substances (sodium arsenate, sodium nitroprusiate) and drugs (bleomicine and statins) either by irradiation (UV, He-Ne laser). The doses and exposure times were chosen as to avoid cell death. After stress removal, cells were allowed to recover and the recovery time period was measured. Structural and functional parameters were evaluated before and after stress, as well as during recovery. By now, experimental models for the in vitro study of non-lethal stress inducing factors have been set up.

P-947

Characterizaton of proteins from human pleural fluid

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The samples of Human Pleural fluid were obtained from both healthy subjects and patients infected by tuberculosis. After the preliminary processing these samples were run in independent lanes of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The two lanes indicated variations in the intensities of a few bands and some new bands were also observed in the infected samples. These were characterized by determining their N-terminal sequences. The new bands which had low density were carefully identified and cloned. Some of the common bands that showed intensity variations were characterized. These were matrix metalloproteins, secretory phospholipaseA_2 , transferrin and ceruloplasmin. They were also studied with MALDI-TOF and their molecular weights have been determined. Some of these proteins have been crystallized and their detailed crystal structure determinations are in progress.

P-948

Chaperones as tools or targets for modulation of pathogenic and therapeutic interventions

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In vivo, heat shock proteins (Hsps) being stress-inducible chaperones can attenuate detrimental consequences of ischemic insults, inflammation, neurodegenerative diseases, etc. Also, intracellular accumulation and chaperone activities of some Hsps may contribute to improved cell survival following UV or ionizing radiation. In models of pathological states and their treatment, we used special virus-based vectors for overexpression of Hsp70 or Hsp27 in cell cultures to confer cytoprotection under simulated ischemia/reperfusion. In parallel, similar cytoprotection was achieved after pretreatments of the cells with a pharmacological Hsp inducer, Geranylgeranylacetone. The cytoprotective effects were manifested in the lesser extent of oxidative modification and aggregation of cellular proteins, better preservation of the cytoskeleton, faster restoration of energy metabolism and the improved post-stress cell survival. In the other model, we treated normal and tumor cells with an inhibitor of the chaperone activity of Hsp90, Geldanamycin. Only the drug-treated tumor cells became more sensitive to gamma-irradiation; such results characterize this drug as a potentially selective radiosensitizer of tumors. Taken together our data demonstrate promising approaches to clinically beneficial manipulating the levels of expression and/or chaperone activity of Hsp(s) by means of gene therapy or pharmacotherapy.

P-949

Probing the bound conformation of Acetylcholinesterase (AChE) inhibitor at the binding site

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Acetylcholinesterases are the enzymes which preferentially hydrolyze acetyl esters (such as ACh or acetyl-â-methylcholine), containing 543 amino acid residues in the *Ee*AChE form and arranged as a 12-stranded â-sheet surrounded by 14 á-helices. The protein is ellipsoidal in shape, with approximate dimensions of 45 Å by 60 Å by 65 Å. Inhibitors of acetylcholinesterase are of commercial and medical interest as pesticides and as therapeutics in the treatment of Alzheimer's disease. An understanding of the conformation of inhibitors in the binding site enables the rational design of novel inhibitors with increased potency and specificity.
Interaction between the ligand, amino-2-methyl-3-(3-trifluoroacetylbenzyl-oxymethyl)quinoline (R414983), and AChE inhibitor has been studied by advanced solid-state NMR through double-quantum chemical shift and distance measurements. Combining solid-state NMR data and docking simulations, conformation of the AChE inhibitor at the active site has been predicted.

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– Drug Design and Delivery –

P-950

Cellular transduction of nucleotide kinases to improve the activation of nucleoside analog prodrugs

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The objective of our study is to improve therapeutic enzyme-prodrug systems by generating catalytically superior nucleoside and nucleotide kinases that are essential for activation of nucleoside analogs. Compounds, such as AZT for the treatment of HIV infections, ACV and GCV used against Herpes virus, or the anticancer compounds AraC and gemcitabine, can enter cells only in the unphosphorylated state (prodrug) and need to be transformed by different kinases to their pharmacologically active triphosphate state that interferes with DNA replication. We have first designed mutants of the human TMP kinase (hTMPK) that phosphorylate AZTMP up to 200-fold faster than wildtype. Expression of this enzyme in human cells leads to 10-fold higher intracellular concentrations of AZTTP and to enhanced HIV inhibition. Second, the prodrugs ACV and GCV are not phosphorylated by human kinases, but are converted to their monophosphate forms by HSV1-TK which is used in enzyme/prodrug-dependent cancer suicide gene therapy. We generated enzyme variants which show selective and efficient phosphorylation of GCV. Third, an engineered human dCK variant catalyzes more efficiently the activation of the prodrugs AraC and gemcitabine. Thus, the concept of a gene (or enzyme) therapeutic treatment involving expression (or direct intracellular transduction) of a catalytically improved human enzyme may pave the way to the development of novel strategies in nucleoside prodrug-dependent cancer chemotherapy.

P-951

Encapsulation of clone vector DNA by cationic diblock copolymer vesicles for gene delivery

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We will discuss the design, control, and structural characterization of cationic copolymer vesicles loaded with DNA. These vesicles serve as a model system for diverse applications such as gene delivery, micro-arraying techniques and packaging of DNA in congested states. Encapsulation of DNA was achieved with a single emulsion technique. For this purpose, an aqueous pUC18 or pEGFP-N1 plasmid solution is emulsified in an organic solvent and stabilized by an amphiphilic diblock copolymer. The neutral block of the copolymer forms an interfacial brush, whereas the cationic block complexes with DNA. A subsequent change of the quality of the organic solvent results in a collapse of the brush and the formation of a capsule. The capsules are subsequently dispersed in aqueous medium to form vesicles and stabilized with an osmotic agent in the external phase. Inside the vesicles, the DNA is compacted in a liquid-crystalline fashion as shown by the appearance of birefringent textures under crossed polarisers and the increase in fluorescence of labeled DNA. The compaction efficiency and the size distribution of the vesicles were determined by light and electron microscopy, respectively, and the integrity of the DNA after encapsulation and subsequent release was confirmed by gel electrophoresis. We demonstrate the gene transfer ability of this new carrier system by the transfection of encapsulated pEGFP plasmid into HeLa cancer cells.

P-952

Characterisation of macromolecular transport in physiologically relevant mixed ECM based gels

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The extracellular matrix (ECM) a complex gel made of hyaluronic acid, collagen and proteoglycans (PG) impedes the penetration of macromolecules especially in tumours, and may compromise the success of novel therapies. Though recent in vivo investigations pointed out that, not only HA but fibrillar collagen its content and organisation and its interactions with PG were involved in macromolecular transport hindrance, transport mechanisms relating the macromolecular drug and these ECM components are unknown.
In this study we seek to evaluate the determinants of passive transport mechanisms of biomacromolecules in complex gels made of HA, collagen using fluorescence techniques (FRAP and confocal reflection microscopy (CRM)) and rheology.
Focus was on conditions relevant to tumours and, initially, on low collagen and relatively high HA content.
Rheology experiments showed that mixed systems containing less than 10mg/mL of HA present higher elastic modulus Ge than pure HA network or pure collagen gels.
Interestingly CRM and FRAP studies revealed similarities for collagen and mixed gels: the organisation and spacing of the collagen fibres did not change and the ratio of the diffusivities (D/D_0) of dextran 2M and IgG were not different but higher than those in HA networks.
Systems with higher collagen content are under investigation to complete the characterisation of transport.

P-953

Understanding the maturation process and inhibitor design of SARS-CoV 3CLpro

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Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel human coronavirus. The viral maturation requires a main protease ($3CL^{pro}$) to cleave the virus-encoded polyproteins. We report here that the $3CL^{pro}$ containing N- and/or C-terminal additional in-frame sequences underwent autoactivation to cleave the tags and yielded the mature protease *in vitro*. The 3-D structure of the C145A mutant protease shows that the active site of one protomer of the dimeric protease is bound with the C-terminal six amino acids of the protomer in another asymmetric unit, suggesting a possible mechanism for maturation. The tagged C145A mutant protein served as a substrate for the wild-type protease and the N-terminus was first digested (55-fold faster) followed by the C-terminal cleavage as shown by the SDS-PAGE analysis. The analysis of the quaternary structures for the tagged and mature proteases by analytical ultracentrifuge experiments reveals the remarkably tighter dimer formation for the mature enzyme than for the mutant (C145A) containing the N-terminal or the C-terminal 10 extra amino acids. Taken together, the study here provides insights to the design of our new structure-based inhibitors.

Posters
– Drug Design and Delivery –

P-954

A New Pulsatile Drug Delivery System

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An ideal drug delivery system should own the following characteristics, the first is the targeting of therapeutic agent to the specific site of their action. The second is the controlled delivery of a therapeutic molecule or protein in a pulsatile or staggered fashion. The third is the achieving sustained zero-order release of a therapeutic agent over a prolonged period of time. In this study, a new drug delivery system combined these characteristics was provided, which contains azobenzene derivatives (AB lipid) as an on-off switch incorporated into liposomes. The drastic release of calcein was observed on the first UV irradiation of AB lipid to the cis isomer, while a suppressed release was observed when irradiated with the first visible light. After that, the slope of release profile became coincident. Furthermore, calcein release was greatly increased after UV irradiation of AB lipid to the cis isomer and the drug release was greatly suppressed after Vis irradiation of AB lipid to the trans isomer. We can control the release rate of calcein from AB lipid/egg PC mixed liposomes by UV or Vis light irradiation.

P-955

A pipeline for similarity and fragment-based docking

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Virtual screening is now widely accepted as a basis for drug discovery thanks to significant improvement and good hit rates [1]. However, it is still highly CPU-consuming. At the same time, the number of protein-ligand complexes described at the atomic level is rising and the sequence similarity is used for structure and function predictions. New approaches are being developed to take advantage of the available structural data and the huge number of protein sequences in order to allow better tuned virtual screening. New web servers are being built to ease and to speed up the whole process (http://abcis.cbs.cnrs.fr/kindock/). Integrating these servers into a pipeline dedicated to molecular modelling (http://abcis.cbs.cnrs.fr/atome/) shall allow both the refined validation of modelled active sites as well as the oriented screening for the primary caracterization of potential ligands.

[1] Cohen-Gonsaud M, Catherinot V, Labesse G, Douguet D (2004) *From molecular modeling to drug design,* **Practical Bioinformatics** 15 (2) *35-72*

P-956

The interaction of the peptide LAH4 with anionic lipids during DNA/RNA delivery to eukaryotic cells

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The histidine rich amphipathic peptide LAH4 has antibiotic and DNA delivery capabilities. The peptide has a strong affinity for anionic lipids found in the outer membrane of bacterial membranes and has shown evidence of higher transfection activity against transformed over healthy tissue in culture. It has been proposed that anionic lipids can flip-flop to reach the cytoplasmic monolayer. Here they neutralise the cationic transfection complexes thereby causing release of oligonucleotides into the cytoplasm. We were, therefore, particularly interested to test for the role of the acidic lipid phosphatidylserine (PS) in mediating LAH4-mediated delivery of DNA efficiency. To understand the potential peptide-lipid interactions in more detail, solid-state NMR experiments on model membranes have been performed. ^{31}P MAS NMR on mixed phosphatidylcholine (PC)/PS and PC/phosphatidylglycerol (PG) membranes has been used to investigate specific LAH4 interactions with anionic lipids. By using deuterated lipids and wide-line ^{2}H NMR when probing lipid chain order, it is demonstrated that LAH4 preferentially interacts with PS over PC. LAH4 thereby effectively disorders the anionic PS lipid fatty acyl chains. The lipid chain destabilising effect of LAH4 and also LAH4 analogues can then be compared with their transfection efficiency for DNA or siRNA in cell culture to aid in rational peptide vector design.

P-957

Docking-molecular dynamics studies on the peroxidase site of prostaglandin endoperoxide H2 synthase

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Prostaglandin endoperoxide H_2 synthases-1 and 2 (PGHS-1 and 2) catalyze the first step in the biosynthesis of prostaglandins, prostacyclins and thromboxanes. Arachidonic acid is transformed into prostaglandin G_2 (PGG_2) at the cyclooxygenase site of the enzyme and the 15-hydroperoxide oxygen-oxygen bond of PGG_2 is subsequently cleaved by reaction with haem at the distinct peroxidase site (POX) to produce prostaglandin H_2 (PGH_2). Herein we present a plausible productive conformation obtained by docking calculations for the binding of PGG_2 to the POX site of PGHS-1. The enzyme-substrate complex stability was verified by a 500-ps molecular dynamics simulation. Structural analysis unveils the requirements for enzyme-substrate recognition and binding: The PGG_2 15-hydroperoxide group is in the proximity of the haem iron and participates in a hydrogen bond network with the invariant His207 and Gln203 and a water molecule, whereas the carboxylate group establishes salt bridges with the remote lysines 215 and 222.

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– Drug Design and Delivery –

P-958

In silico elucidation of xenobiotic processing loops

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One of the important challenges for drug designers is to predict and analyze how drugs are absorbed, distributed, metabolized and excreted (ADME) in the body. These processes highly correlated with toxicity of drugs and are actively studied in pharmacology. Two classes of proteins, the drug metabolizing enzymes such as cytochrome P450s (CYPs) and transporters, are the target of such ADME/Tox research. It was relatively recent that these two classes of proteins are synthesized by the genes that are the target genes of the nuclear receptors. Nuclear receptors are ligand-activated transcription factors that form a superfamily. In case of humans there are 48 nuclear receptors almost half of whose ligands are identified, leaving some as true orphans. Thus it was now recognized that these nuclear receptors play the role of sensors of drugs and other xenobiotic substances including environmental chemical pollutants and nutritional ingredients, while the drug metabolizing enzymes and the transporters are the processors which carry the actual cleaning jobs. We have started to elucidate the feedback loops that are formed by the xenobiotic ligands, nuclear receptors, their target genes, their product proteins, and their feedback actions on the ligands. The work is being carried out on our background database on the ligands and their receptors called KiBank, and search programs for target genes of nuclear receptors algorithmically. The most recent results will be presented at the presentation.

P-959

Membrane electroporation - Tool for therapeutic electrotransfer of drugs and gene DNA

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Membrane electroporation (MEP) is a new electrical high voltage scalpel, transiently opening the cell membranes of tissue for the penetration of foreign substances. Due to the enormous complexity of cellular membranes, many fundamental problems of MEP have to be studied at first on model systems, such as the curved bilayer membranes of unilamellar lipid vesicles.
Electrooptical and conductometrical data of unilamellar liposomes indicate that electric field pulses cause not only the formation of membrane electropores but also shape deformation of the liposomes, both processes mutually affecting each other. The primary field effects of MEP and cell deformation can trigger a cascade of numerous secondary phenomena, such as pore percolation and transport of small and large molecules across the electroporated membrane. The chemical MEP theory represents a molecular physico-chemical approach to electrochemomechanical pore formation, yielding transport parameters, such as permeation coefficients, pore fractions and pore sizes. The pore concept is successfully applied to rationalize optimization strategies for biotechnological and medical applications of MEP.

P-960

Controlled degradability of polysaccharides multilayer films *in vitro* and *in vivo*

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Polyelectrolyte multilayer films (PEM) adsorbed on biomaterial surfaces are a new way to create a controlled release system. Using biodegradable polymers, the films can be degraded *in vivo* and release active molecules. In this work, we demonstrate the possibility of tuning the degradability of polysaccharide PEM *in vitro* and *in vivo*. Chitosan and hyaluronan PEM (CHI/HA) were either native or cross-linked (CL) using a water soluble carbodiimide (EDC) at various concentrations in combination with N-hydroxysulfosuccinimide. The *in vitro* degradation of the films in contact with enzymes was followed by quartz crystal microbalance measurements and confocal laser scanning microscopy after film labeling with CHI^{FITC}. Whereas the native films were subjected to degradation, the CL films were more resistant to enzymatic degradation. Films made of chitosan of medium molecular weight were indeed more resistant than films made of chitosan-oligosaccharides. In addition, macrophages could degrade all types of films and internalize the chitosan *in vitro*. The native films implanted *in vivo* in mouse peritoneal cavity for a week showed an almost complete degradation whereas the CL films were only partially degraded. These results suggests that the polysaccharides PEM are of potential interest for *in vivo* applications as biodegradable coatings and that degradation can be tuned by controlling film cross-linking.

P-961

Tryptophanase inhibitors:towards a new generation of antibiotics eliminating biofilm formation

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Tryptophanase (Trpase), a bacterial enzyme with no counterpart in eukaryotic cells, produces L-Trp pyruvate ammonia and indole. It was suggested that indole is essential for bacteria multiplication and biofilm formation. Biofilms destroy equipment and food and cause many illnesses. Most synthesized quasi-substrates inhibit Trpase at mM range. An optimal and specific inhibitor of Trpase may eliminate indole production and prevent biofilm formation. X-ray crystallography of the holo-WT *E. coli* Trpase soaked with L-Trp and the known mechanism of Trpase activity should provide the information for the design and synthesis of active-site-specific quasi-analogs. Utilizing the chromogenic substrate S-(*o*-nitrophenyl)-L-cysteine, the following Michaelis-Menten kinetics analyses determined the mode of Trpase inhibition by Trp and quinone based quasi-analogues and the corresponding Ki values (in μM): dl-2-Alanyl-2,10 anthraquinone, noncompetitively, 110; Trypthophan ethylester, competitively, 24; acetyltryptophan, uncompetitively, 61.5; S-phenylbenzoquinone-L-tryptophan, uncompetitively, 92. PLP-L-Trp, inhibited irreversibly only the apo form of Trpase and may serve for structure-determination purposes. Further attempts are being made to synthesize improved Trpase inhibitors, i.e., in the nM range.

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– Drug Design and Delivery –

P-962

Design of peptides with consecutive dehydro phenylalanine residues

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In order to develop general rules for the design of peptide conformations with consecutive alpha, beta-dehydro phenylalanine-residues, peptides were synthesized, crystallized and crystal structures and molecular conformations were determined. Following conclusions were drawn based on the structural data:
— peptide unit sequences with two consecutive dehydro-Phe residues at (i+1) and (i+2) positions adopt an unfolded S - shaped structure with dihedral angles phi-psi centred at $\pm 60°$, $\pm 30°$.
— the peptides containing two consecutive dehydro-Phe residues at (i+2) and (i+3) positions
• form two overlapping type III beta - turns (incipient 3_{10} - helix).
• with branched beta - carbon residue only at (i+1) position adopt a conformation with two overlapping types II and III′ beta - turns.
• with branched beta- carbon residues such as Val and Ile at both (i+1) and (i+4) positions form two overlapping types II and I′ beta - turns.
The consistency in the formations of these conformations makes the design of peptides with alpha,beta ?- dehydro - residues a useful and highly predictable method for developing specific ligands for various biological applications including drug design.

P-963

One-Step Preparation of Liposomes Loaded with Doxorubicin by Ethanol Injection Method

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Liposomes offer new approaches for drug delivery through their encapsulation to alter pharmacodynamic properties of loaded drug leading to reduction in toxicities and/ or improved efficacy. For prolonged systemic circulation, the liposomes size has been shown to be limited to 200 nm or less. The ethanol injection method is an excellent technique for the formation of liposomes of < 200nm without the need of sonication or extrusion. The present study was aimed to produce liposomes encapsulating doxorubicin in minimum procedural steps. Liposomes were prepared using distearoyl phosphatidylcholine and cholesterol, distearoyl phosphatidylcholine, cholesterol and oleic acid. The effects of different operational conditions for vesicle production and drug encapsulation were evaluated, with a view to achieve process cost to a minimum, suitable size and high encapsulation efficiency. Although high efficiency of doxorubicin encapsulation was obtained by 'active' or 'remote' loading process in DSPC/Chol system, it was poor in one-step injection method. Oleic acid was included to cut down the active loading by pH-gradient. DSPC/Chol/OA systems spontaneously loaded doxorubicin with encapsulation efficiency of ~ 50 % and final drug to lipid molar ratio upto 0.172. The mean diameter of the vesicles was 175 ± 5 nm. The method offers liposomes of small size with high loading.

P-964

Interplay between polymerized liposomes physicochemical properties and composition and citotoxicity

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This study was aimed at investigating whether there is an interplay between diacetylenic polymerized liposomes physicochemical properties and lipid composition affecting citotoxicity *in vitro*. Unsaturated 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine with saturated 1,2-dimiristoyl-sn-glycero-3-phosphocholine in molar ratio 1:1, were combined to give a chemically modified membrane by UV-polymerization. Biophysical characterization was carried out determining the hydrophobic factor and hydrodynamic radius. Citotoxicity was evaluated through haemolytic capacity on bovine red blood cells and indirectly by capacity of induction of lipid peroxidation on microsomes or mitochondrial membranes. The haemolysis percentage in presence of DC8,9PC/DMPC is less than that induce by polymers used in dentistry. The data obtain suggests that the polymerized lipids can not induce lipid peroxidation on natural membranes. The polymerized diacetylenic liposomes showed less interaction with serum proteins than non polymerized and lower citotoxicity as compared with natural lipids. Also cell viability was determined in cell line NIH3T3 after exposure to lipids systems under study.
The hydrophobic factor showed further augmentation for polymerized liposomes and is discussed in relation to *in vitro* stability. The above results suggest that polymerized and non-polymerized liposomes would serve as an effective delivery vehicle.

P-965

Influence of pH and lipid composition on lipid bilayer permeation of small aromatic carboxylic acids

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The major route for drug entry into cells is permeation across lipid bilayers. Due to methodological limitations there are only few studies on permeation of drug-like molecules across lipid bilayers. An assay developed in our lab allows the direct measurement of lipid bilayer permeation of aromatic carboxylic acids (ACAs). Tb^{3+}, which forms a fluorescent complex with ACAs, is entrapped in liposomes and ACA entry is determined from luminescence increase. Lipid bilayer permeation was *pH*-dependent, following a Henderson-Hasselbalch function with a plateau for the neutral and the anionic species, respectively. In contrast to the expectations of the *pH*-partition hypothesis, permeation of the anionic species was only 1 to 3 magnitudes lower than that of the neutral species, leading to anion-controlled permeation at *pH* 7.4, independently of bilayer state and lipid composition.
Permeation across bilayers with a biologically relevant lipid composition was significantly slower than across egg-phosphatidylcholine membranes. The influence of single lipids, such as cholesterol, was dependent on the structure and ionization state of the permeant.
Permeation coefficients of the neutral species correlated better with the polar surface area (PSA) than $\log P_{oct}$, therefore PSA is a better predictor for bilayer permeation of the neutral species of small ACAs than $\log P_{oct}$.

Posters
– Drug Design and Delivery –

P-966

A specific non-competitive inhibitor of a small G protein/GEF complex

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Abnormal activation of small G proteins is involved in several human diseases. Small G proteins are activated by GDP/GTP exchange, which is stimulated by their guanine nucleotide exchange factors (GEFs). Thus, small G protein/GEF complexes appear as emerging targets for interrupting signalling networks regulated by small G proteins in pathological contexts. The activation of small G proteins of the Arf family is initiated by exchange factors which carry a Sec7 catalytic domain stimulating the dissociation of the bound GDP nucleotide. The structure of the Arf1-GDP-ARNO reaction intermediate, trapped by a mutation of the catalytic glutamate, was recently solved by X-ray crystallography (PDB code: 1R8S). Using this structure, a small molecule inhibitor was identified by virtual screening, and was shown to inhibit the exchange reaction *in vitro*. The results of this study and the kinetic characterization of this inhibition will be presented.

P-967

Prediction of the Binding Free Energies for HIV-1 Integrase with Its Inhibitors with LIE Method

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Abstract In this work, the Linear Interaction energy (LIE) method was used to calculate the binding free energies of HIV-1 Integrase (IN) and a series of dicaffeoyl - or digalloyl pyrroliding and furan derivatives inhibitors. The model of binding free energy prediction for homogeneous inhibitors of HIV-1 IN has been obtained with a root-mean-square deviation (RMSD) of 1.39 kJ/mol and estimated to be a precise model with good prediction capability. In addition, the probable binding mode of this series of inhibitors with HIV-1 IN was proposed by using molecular docking and molecular dynamics (MD) simulation methods. Our results indicate that caffeoyl - or galloyl group of inhibitors have close interaction with a HIV-1 IN conservative DDE motif.

P-968

Protein crystallization and structural study of uPA protease domain with active site serine mutation

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The urokinase (uPA) system is composed of uPA, its receptor (uPAR), and inhibitor (PAI). It plays important role in various physiologic processes, including fibrinolysis, cell adhesion, and signal transduction, and has been recognized as a target for intervention in tumor growth and tumor metastasis. We constructed an active site mutant of uPA protease domain (159-405) with three mutations (C279A, N302Q, and S356A) and expressed it as secreted protein in pichia pastoria with pPICZa vector. The secreted mutant was captured from culture medium by a cation exchange column and then further purified on a gel filtration column. The purified mutant was then crystallized by sitting drop vapor diffusion method with several precipitant conditions: (1) 1.5-2.1M ammonium sulphate, 5-8% PEG400, 50mM sodium citrate pH 4.6 or 50mM sodium phosphate pH 7.5, 0.05% sodium azide. (2) 1.8M ammonium sulphate, 0.14M Lithium sulphate, 50mM sodium acetate at pH 5.2, (3) 2.5-2.8M sodium formate, 50mM sodium acetate at pH 5.2. (4) 3.2-3.6M sodium chloride, 50mM sodium acetate at pH 5.2. The crystals were of varying quality but generally diffracted from 1.7Å-2.1Å with in-house X-ray source. The structure of this uPA mutant and its complex with various inhibitors will provide a platform for rational uPA inhibitor design.

P-969

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

– Drug Design and Delivery –

P-970

On the protective role of selenium and catechin in cadmium toxicity

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Cadmium as heavy metal is toxic and carcinogenic for organisms. Cadmium perform their effects on living organisms by accumulation in blood and various tissues. Due to their accumulation in various tissues and in blood, tissue antioxidant enzyme systems are affected. The present study was planned to determine the possible protective roles of selenium and catechin against the toxic effects of considered heavy metals. The study has been performed in Wistar Albino type rats which divided into four groups as control and cadmium, cadmium+selenium, cadmium+catechin received groups. Besides cadmium as heavy metal, selenium concentration determinations were performed in blood, liver and kidney tissues of each group of rats. In the same tissue samples besides lipid peroxidation measurements, glutathione, glutathione peroxidase and superokside dismutase enzyme activity determinations were also performed. The accumulation of heavy metals was determined in blood, liver and kidneys after cadmium administration during experimental period. In the tissue of experimental group animals there was an increased lipid peroxidation but decreased antioxidant enzyme activities were observed. While effects of selenium in decreased toxicity of cadmium have been detected, there was no statistically significant effect of catechin observed.

Posters
– Rotors and Motors –

P-971

DSC study on the motor protein myosin

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The force generation in muscle arises from direct interaction of the two main protein components of the muscle, myosin and actin. The process is driven by the energy liberated from the hydrolysis of ATP by myosin. The interaction is performed by cyclic interaction of myosin with ATP and actin, and at least six intermediates are proposed for actomyosin ATPase in solution.
The powerful DSC technique allows the derivation of heat capacity of proteins as a function of temperature. From the deconvolution of the thermal unfolding patterns it is possible to characterize the structural domains of the motor protein. In this work we tried to approach the temperature-induced unfolding processes in different intermediate state of ATP hydrolysis in striated muscle fibres. We have extended the experiments to study the fiber system prepared from psoas muscle of rabbit in rigor, strongly binding and weakly binding states of myosin to actin where the inorganic phosphate (P_i) was substituted by the phosphate analogue orthovanadate. The DSC transitions were analyzed in different buffer solutions (Tris and MOPS) to get information about the temperature dependence of pH on the conformational changes.

P-972

Engineering a bio-molecular walker

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In this work we describe the design and development of a bio-molecular walker based on the motile system found in certain ciliated protists. The motile system is driven by the binding of Ca^{2+} ions and in contrast to other commonly studied motor proteins is independent of ATPase (Amos et al., 1975). The motor protein is a 20kDa Ca-binding protein called spasmin (Maciejewski et al.,1999; Itabashi et al., 2003) which belongs to the EF-hand family of calcium binding proteins called calmodulins. Upon calcium binding, spasmin is thought to undergo a large conformational change as it binds its own target peptide and we wish to exploit this in order to create our own novel molecular walker.We have created a recombinant spasmin with sequence tags to enable specific immobilization and various conjugate chemistries. Cystein mutants have been introduced at specific points in the protein to enable attachment of other small molecules, for example fluorophores. We are now optimising protein expression and purification to maximise the yield of active protein on which we can perform the conjugate chemistries. We will characterize the structural changes using biophysical methods such as circular dichroism, analytical ultracentrifugation, electron microscopy, AFM and total internal reflection fluorescence spectroscopy on single molecules. *[This collaborative work is funded by the Koerber Stiftung, European Science Award 2003]*
Amos W. B. *et al. J. Cell Sci.* 19: 203-213 (1975), Maciejewski J. J. *et al J.Euk.Microbiol* 46:165-173 (1999), Itabashi T. *et al. Research in Microbiology* 154:361-367 (2003)

P-972-B

Kinesin and dynein move a peroxisome *in vivo*: a tug-of-war or coordinated movement?

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We have used Fluorescence Imaging with One Nanometer Accuracy (FIONA) for analysis of organelle movement by conventional kinesin and cytoplasmic dynein in a cell. We can locate a green fluorescence protein (GFP)–tagged peroxisome in cultured *Drosophila* S2 cells to within 1.5 nanometer in 1.1 milliseconds, a 400-fold improvement in temporal resolution, sufficient to determine the average step size to be $\sim$8 nanometers for both dynein and kinesin. Furthermore, we find that dynein and kinesin do not work against each other in vivo during peroxisome transport. Rather, we find that multiple kinesins or multiple dyneins work together, producing up to 10 times the in vitro speed.

P-973

Cooperative extraction of membrane nanotubes by molecular motors

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In eukaryotic cells, nanotubes represent a substantial fraction of transport intermediates between organelles. They are extracted from membranes by molecular motors walking along microtubules. We previously showed that kinesins fixed on giant unilamellar vesicles in contact with microtubules are sufficient to form nanotubes *in vitro*. Motors were attached to the membrane through beads, thus facilitating cooperative effects. Koster *et al.* [Kosteret al. (2003) *PNAS USA*] proposed that motors could dynamically cluster at the tip of tubes when they are individually attached to the membrane. We demonstrate, in a recently designed experimental system, the existence of an accumulation of motors allowing tube extraction. We determine the motor density along a tube by using fluorescence intensity measurements. We also perform a theoretical analysis describing the dynamics of motors and tube growth. The only adjustable parameter is the motor binding rate onto microtubules, which we measure to be 4.7 +/- 2.4 s^{-1}. In addition, we quantitatively determine, for a given membrane tension, the existence of a threshold in motor density on the vesicle above which nanotubes can be formed. We find that the number of motors pulling a tube can range from four at threshold to a few tens away from it. The threshold in motor density (or in membrane tension at constant motor density) could be important for the understanding of membrane traffic regulation in cells.

Posters
– Rotors and Motors –

P-973-B

A dimeric 1-d lattice gas as model for molecular motors collective dynamics

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The transport of molecular motors along microtubules closely resemble the dynamics of a driven lattice gas of dimers without conservation of particles.
The unidirectionality, asymmetry and stochasticity of the motion are encoded in the well studied Totally Asymmetric Simple Exclusion Process (TASEP). We extend the model to a more realistic one, including attachment and detachment kinetics and extended (dimeric) particles.
We study the stationary phase diagram by means of Monte Carlo simulations combined with a continuum description (based on an extended mean field theory). We also evaluate the domain wall theory finding out the effective potential confining the phase interface into the bulk.

P-974

Optical trap with fast programmable feedback loop to study rotary molecular motors

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An optical trap with back-focal plane detection and fast programmable feedback has been developed for the study of rotary molecular motors. A helium-neon laser (632 nm) is used for position detection and a solid state fibre laser (1064 nm, 3W CW) forms the trap. Acousto-optic deflectors (AODs) controlled by a digital signalling processing board are used to achive programmable feedback loops with flexible control options and speeds up to 8 kHz. Several modes of feedback are demonstrated, controlling both bead position (x,y) and angle (r, θ). Polystyrene beads or bead pairs can be held at set (x,y) or θ, and the set-point can be changed while the program is running. For example, feedback can be used to move a bead or a bead pair in a circle. Results of using the system to study the bacterial flagellar motor are presented.

P-975

Movement of coupled single-headed kinesins analysed by a Brownian-ratchet model

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The movement of truncated single-headed kinesins attached to a sub-micrometer bead was observed recently by Kamei et al. [Biophys. J. **88** (2005) 2068]. The analysis of this system is expected to provide insights into the mechanism underlying the motility of conventional double-headed kinesin, espetially the roles played by individual heads. We would like to clarify whether the experimentally observed behaviors that are supposed to be caused by a pair of single-headed kinesins can be explained by a simple Brownian-ratchet model, which is successful in describing the motion of an unconventional single-headed kinesin KIF1A. Our model consists of two Brownian motors (ratchets) separated by a fixed distance *r*. The velocity and other quantities of the coupled motors are calculated by solving the Fokker-Planck equation with various choices of *r* and other parameters. Then, assuming a certain probability distribution of *r* associated with random attachment of kinesin heads on a bead in the experiment, the statistical properties of the motion of the coupled Brownian motors are analysed. The force-velocity relation observed experimentally is found to be consistent with the present model with appropriate choices of the model parameters. The adequacy of the parameter choice needs to be confirmed by other experiments.

P-976

Single kinesin motor proteins walking through the searchlight

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The dimeric motor protein kinesin steps by a hand-over-hand mechanism. This means that the centre of mass moves with 8 nm steps, while the two motor domains, the one after the other, move 16 nm to the next binding site on the microtubule. The molecular details of what happens during a step are not fully understood, partly because of lack of time resolution in wide-field, single-molecule fluorescence experiments. We set out to develop an approach to study the motility of kinesin with a time resolution below a millisecond (a single step takes on the order of 10 milliseconds). This approach allows us to look into the mechanochemistry and coupling of the two kinesin motor domains while they are stepping. Our method is based on confocal microscopy and we study the fluorescent properties of single labeled motors while they walk through the confocal laser spot. We present the experimental details of our approach and show our results on human kinesin constructs that are specifically labeled in the tail. We show that our approach enables us to study the mechanism of kinesin with a much higher time resolution than what was achieved before with single-molecule fluorescence experiments.

Posters

– Rotors and Motors –

P-977

Stochastic cooperativity model for molecular motors

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Molecular motors often consist of two or more subunits that cooperate to convert chemical energy into mechanical motion. Hexameric helicases and viral packaging ATPases constitute a special class of molecular motors that translocate along nucleic acids. Recent structural and spectroscopic characterization of these motors revealed that their enzymatic cooperativity does not result from cooperative binding [1-2]. In order to understand this new type of cooperativity we simulated the kinetics of a single hexameric motor by multiple coupled stochastic reactions using the Gillespie algorithm [3]. In contrast to analytical methods, our direct simulation allowed us to investigate the kinetics with an arbitrary model for cooperativity between the subunits. Simulations on the kinetics of the hexameric RNA packaging motor P4 from dsRNA bacteriophage [1] with different cooperativity mechanisms provided insight into the RNA-mediated cooperativity and yielded a sound theoretical basis for the interpretation of experimental results [2].

[1] Mancini, E. J., et al, Cell 118, 743-755, 2004
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Posters
– The RNA World –

P-978

RNA structures of HIV-1 dimerization initiation site in the kissing-loop and extended-duplex dimers

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Generation of RNA dimeric form of the human immunodeficiency virus type 1 (HIV-1) genome is important for the viral replication. The dimerization initiation site (DIS) has been identified as a short sequence that can form a stem-loop structure with a self-complementary sequence in the loop and a bulge in the stem. A 39mer DIS RNA fragment, DIS39, spontaneously formed "loose-dimer" and was converted into "tight-dimer" by supplement of nucleocapsid protein NCp7. NMR chemical shift analysis for DIS39 in the kissing-loop and extended-duplex dimers revealed that three dimensional structures of the stem-bulge-stem region were similar between the two types of dimers. Therefore, we determined the solution structures of two shorter RNA molecules corresponding to the loop-stem region and the stem-bulge-stem region of DIS39, and the solution structures of DIS39 in the kissing-loop and extended-duplex dimers were determined by combining the parts of structures. The mechanism of conformational conversion will be discussed based on the solution structures and the molecular dynamics analysis.

P-979

Mechanism of hairpin-duplex conversion for the HIV-1 Dimerization Initiation Site

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We have used the Dimerization Initiation Site (DIS) of HIV-1 genomic RNA as a model to investigate hairpin-duplex interconversion by using a combination of fluorescence, UV-melting, gel electrophoresis and X-ray crystallographic techniques. Fluorescence studies with Molecular Beacons and crystallization experiments with 23-nucleotide DIS fragments showed that the ratio of hairpin to duplex formed after annealing in water essentially depends on RNA concentration, and not on cooling kinetics. With natural sequences able to form a loop-loop complex (or 'kissing complex'), concentrations as low as 3 μM in strands are necessary to obtain a majority of the hairpin form. On the contrary, when kissing-complex formation was made impossible by mutation in the loop, a majority of hairpins was obtained even at 80 μM in strands. This mutated sequence also showed that kissing-complex formation is not a prerequisite for an efficient conversion to duplex in presence of salts. We proved that this happens through hairpins engaged in a cruciform intermediate, but not from free strands after hairpin melting. Supporting this view, the very first step of formation of such a cruciform intermediate could be trapped in a crystal structure. Such a mechanism might be biologically significant beyond the strict field of HIV-1 RNA dynamics.

P-980

Characterisation of the interactions between HIV-1 VIF and genomic RNA: a fluorescence study

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The Viral Infectivity Factor (Vif) encoded by HIV-1 is a small basic protein that strongly modulates the viral replication and is required for pathogenicity. Vif is packaged into HIV-1 particles through a strong interaction with genomic RNA and is associated with viral nucleoprotein complexes. Moreover Vif acts during the early stages of the viral infection (capsid disassembly, reverse transcription) as well as during the late stages of virus replication (virus assembly and maturation of the virion). However the effect on early stages is probably a consequence of a defective assembly and / or virion maturation. Understanding the RNA-binding properties of Vif would contribute to elucidate the role played by Vif in the regulation of the genomic RNA trafficking in the cytoplasm to unable efficient packaging, and the prevention of cellular inhibitors from altering HIV-1 RNA. In this context, we have characterised the interactions of recombinant Vif with HIV-1 genomic RNA by fluorescence spectroscopy, and determined the affinity of the protein for synthetic RNAs corresponding to various regions of HIV-1 genome. Taken together our results demonstrate cooperative and specific binding. In particular, we showed that Vif has a high affinity for the 5'-untranslated region of HIV-1 genomic RNA.

P-981

Domain arrangement in the SRP protein Ffh from E. coli as determined by FRET

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The signal recognition particle (SRP) initiates the co-translational targeting of proteins to the plasma membrane in bacteria by binding to the N-terminal signal sequence emerging from the translating ribosome. SRP in *Escherichia coli* is composed of a 48-kDa protein, called Ffh, and a 114-nucleotide RNA, called 4.5S RNA. The crystal structures of NG and M domains of Ffh and of the complex of the M domain with the RNA are known, wheras the domain arrangement in free and RNA-bound Ffh are not. We have probed the structure of *E. coli* Ffh and its complex with 4.5S RNA by fluorescence resonance energy transfer (FRET), measuring distances between fluorophores at different positions within Ffh and between Ffh and 4.5S RNA. According to the FRET distances, NG and M domains in free Ffh are closely aligned resembling the A/A arrangement in the crystal structure of Ffh from *Thermus aquaticus*. Upon Ffh binding to 4.5S RNA the G and M domains move apart to assume a more open configuration, as indicated by changes of FRET distances. In SRP, 4.5S RNA seems to be present in a bent, rather than extended, conformation. The domain arrangement in both free and RNA-bound Ffh differs from that in the crystal structure of the homologous *Sulfolobus solfataricus* SRP54 and its complex with a fragment of 4.5S RNA.

Posters
– The RNA World –

P-982

Modeling the long range entropy of RNA:

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Non-coding RNA appears to make up a large part of the human genome. A reliable RNA structure prediction program is needed to understand the structure of this non-coding RNA.
We recently developed a new way to model the long range entropy in RNA and applied it to RNA secondary structure prediction. In some of instances, the new approach is able to achieve far better predictions than the state of the art secondary structure programs even given exactly the same parameters. Predictions using this method tend to show distributions that are funnel shaped. A new and important parameter in these calculations is the persistence length (a measure of the correlation and flexibility of the RNA). (URL: http://www.rna.it-chiba.ac.jp/∼vsfold/vsfold4/)
This new approach has now been extended to prediction of pseudoknots. The method is a heuristic wherein the hierarchical folding hypothesis is used to find the pseudoknots as the RNA secondary structure is folding, and corrections to that secondary structure are made to accommodate the pseudoknot. It is able to do these searches in roughly N^4 time. The model is consistent with the hierarchical hypothesis and it is possible to estimate RNA folding times that are of the correct order of magnitude using this model.
With further adaptations to account for the size, shape and variablity of amino acid residues (hydrophobicity etc.), the model also appears to be transferable to protein folding problems.

P-983

Aminoglycoside binding to HIV-1 DIS kissing-loop complex: from crystals to cells

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All retroviral genomes consist in two homologous single stranded RNAs. Dimerization is an essential step for viral replication. HIV-1 Dimerization Initiation Site (DIS) is a strongly conserved stem-loop in the 5' leader region of the genomic RNA. It was shown *in vivo* that alteration of the DIS dramatically reduces viral infectivity. We have previously solved crystal structures of the DIS kissing-loop complex. Analysis of these structures revealed an unexpected resemblance between the DIS kissing-loop and the 16 S ribosomal aminoacyl-tRNA site (A-site), which is the target of aminoglycoside antibiotics. We have shown that some aminoglycosides specifically bind to the DIS kissing-loop complex with an affinity and geometry similar to that observed in the A-site.
In agreement with these previous results, we have now solved high-resolution crystal structures of the DIS kissing-loop complex bound to four aminoglycosides. These structures show that, as expected, two aminoglycosides are bound per kissing-loop complex. Importantly, the binding is observed not only *in vitro* on large HIV-1 genomic RNA fragments, but also on infected cells. Moreover, we showed that some of these aminoglycosides stabilize the kissing-loop RNA dimer, which is consistent with the observation in crystal structures of numerous direct and water-mediated drug-RNA contacts. These structures are currently used as starting points for designing potential new drugs targeted against the viral RNA.

P-984

NMR and molecular modelling studies of an RNA hairpin containing a G-rich hexaloop

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The mRNA of the PGY1/MDR1 gene encoding the transmembrane P-glycoprotein (P-gp) contains a hairpin that is the target of antisense oligonucleotides, suppressing the P-gp function of multidrug resistance. The solution conformation of this hairpin constituted by the 5'(GGGAUG)3' loop closed by a G-U mismatch containing stem is studied by NMR and molecular dynamics in explicit solvent. Special attention is given on the sugar and the backbone conformations and the hexaloop intrinsic properties of these two components are carefully investigated. The stem structures obtained by molecular dynamics with and without NMR constraints converge to the same A-type double helix. The wobble G-U mismatch moderately perturbs the overall conformation, despite of C2'-endo sugars and unusual backbone conformations located between the mismatch and the loop. In the hexaloop part, the sugar puckers are in majority in C2'-endo conformations, probably to extend the strand with the help of unusual backbone angles conformations. The loop appears stabilized by one hydrogen bond and stacking interactions. Thus, from the 5' to the 3'- ends, the four purine bases GGGA are stacked together, then a U-turn like is observed, and finally, U stacks on the last G that remains rather far from the stem.

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Structural basis for the antigene and antisense properties of modified DNA:DNA and RNA:DNA duplexes

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Oligonucleotides containing polyamines are currently being evaluated as potential antigene and antisense compounds. Those with 5-(*N*-aminohexyl)carbamoyl-2'-deoxyuridine (^{N}U) and its 2'-*O*-methyl derivative ($^{N}U_m$) exhibit improved nuclease resistance and form stable duplexes with their DNA and RNA targets. X-ray structures of these duplexes have shown good correlation between the conformational changes and the observed chemotherapeutic properties.
The amide groups of the modified uracil bases form six-membered rings through the intra-molecular NH—O4 hydrogen bonds, so that the aminohexyl chains protrude into the major grooves. Some of the terminal ammonium groups are involved in intra-duplex interactions with phosphate oxygen anions, whereas the others interact with those of the adjacent duplex. Such interactions contribute to the stability of duplex formation. The 2'-*O*-methyl modification in $^{N}U_m$ shifts the ribose ring toward the C3'-*endo* conformation and influences duplex stability. Observed changes in the dimensions of the minor grooves and in the hydration structures are also well correlated to nuclease resistance and duplex stability.

P-987

Insights into the splice site formation of group II intron ribozymes

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Most RNAs undergo several steps of post-transcriptional modification before carrying out their assigned functions. One of the major modifications is the splicing process, by which non-coding introns are removed from the coding exons. Splicing can be performed by autocatalytic, self-splicing introns (e.g. group II introns), i.e. catalytic RNA or ribozymes. Group II introns, which occur in bacterial genomes and in organellar genes of plants, funghi and lower eukaryotes, consist of a conserved set of six domains. Domain 1 recognizes the 5'-exon through a 10-15 base pairing interaction formed by two regions within the intron (exon binding sites, EBS1 and EBS2) and the last 10-15 nucleotides of the 5'-exon (intron binding sites, IBS1 and IBS2). As the correct recognition of IBS1 by EBS1 is crucial for a successful splicing event we are investigating the structural and metal ion requirements of this part by various spectroscopic techniques, e.g. NMR. Our data shows that the hairpin including EBS1 consists of a helical region followed by an unstructured single stranded part, which is ready for splice site recognition. The results of the structure analysis will be presented.
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Structural basis for substrate binding, cleavage and allostery in the tRNA maturase RNase Z

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Transfer RNAs are synthesized as part of longer primary transcripts that require processing of both their 3' and 5' extremities in every living organism known. The 5' side is matured by the quasi-universally conserved endonucleolytic ribozyme, RNase P, while removal of the 3' tails can be either exonucleolytic or endonucleolytic. The endonucleolytic pathway is catalysed by an enzyme known as RNase Z. RNase Z cleaves precursor tRNAs immediately after the discriminator base in most cases, yielding a tRNA primed for addition of the CCA motif by nucleotidyl transferase. RNase Z is found in the vast majority eukaryotes and archaea and in about half of the sequenced bacteria. It is often essential for growth and mutations in one of the two genes encoding RNase Z (ELAC2) have been linked with prostate cancer in man. In this poster we present the crystal structure of Bacillus subtilis RNase Z at 2.1Å resolution (1) resolved by MAD method and propose a model for tRNA recognition and cleavage. The structure explains the allosteric properties of the enzyme and also sheds light on the mechanisms of inhibition.It also highlights the extraordinary adaptability of the metallohydrolase domain of the b-lactamase family for the hydrolysis of covalent bonds.
(1)I. Li de la Sierra-Gallay, O. Pellegrini & C. Condon (2005). Nature 433:657-661

P-989

Coformational language of proteins as a possible mechanism of the replicators evolution

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A model of the genome as a gene network capable of receiving information about the environment and performing some operations on genes has been considered. The evolution rate of replicators for the mechanism of random mutations has been estimated. It was shown that the evolution rate under random actions is negligibly small for real dimensions of genomes of replicators [1]. It was inferred that only a deterministic mechanism of the evolution can explain the known evolution rate of replicators. A deterministic model of the evolution has been proposed. The basic principles of this model include: 1) Information about the replicators evolution is encoded in the conformational states of proteins; 2) The conformational language of proteins is translated into the language of nucleotide sequences during the evolution; 3) The structure of genes is controlled such that the transition to a nearest free ecological niche takes a minimum time (at a preset restriction on the control).
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Context-dependent selection of promoter in a natural selection-type evolution reactor

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Using an isothermal amplification of hairpin DNA/RNA (developed by G.Joyce), we drove a natural selection-type evolution reactor taking the specific growth rate as the fitness. We used HIV-1 RT and ThermoT7 RNAP at 50°C. Starting from the random promoter pool, we selected the strongest promoter at 50°C, which was separated by Hamming distance 2 from the strongest promoter at 37°C. The latter was found to be identical to the natural T7 promoter. When we used a simple random pool, the selection process showed one-step convergence. When we used a random pool with a specific short sequence at the upstream flanking region of the random region, we observed an evolution process as convergence-divergence-convergence of the promoter sequence, driven by deletion of the specific short sequence. This context-dependent selection was found to come through a neutral path, judged from the fitness measurement.

P-991

Structural properties of CTG/CAG repeats, and preliminary X-ray analysis of CUG repeats

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The human genome contains so many different types of repetitive sequences. Some of them are tandem repeats of trinucleotides. Their unusual expansions cause genetic diseases such as type 1 myotonic dystrophy (DM1) and Huntington's disease (HD), the unit sequences being CTG and CAG, respectively.
The numbers of repeats of the two complementary sequences change independently during DNA replication or repair. The direct origin of DM1 is, however, the transcribed RNA fragments with CUG repeats, which forms a specific structure and inhibits other protein syntheses. In the present study, structural versatilities of such DNA and RNA fragments has been examined.
Native PAGEs of $(CUG)_n$ show that the hairpin structure with even number is more stable than that with odd number. This difference might be ascribed to the structural difference at the hairpin head. The PAGEs also show that duplex formation is dependent on coexisting cationic species and their concentration. Crystal data of $(CUG)_6$ (a=b=39.6, c=141.0 Å, and the space group $R32$) suggest that the asymmetric unit contains the RNA fragment. An approximate crystal structure solved by molecular replacement techniques at 1.95 Å resolution shows that the RNA fragments form a duplex similar to an A-form RNA.

P-992

Modified nucleosides and across the anticodon loop interactions in tRNA

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In several interesting tRNA molecules, the (34^{th}) as well as the (37^{th}) nucleoside are hyper modified. As an example, unique hypermodified nucleosides $mcm^5s^2U_{34}$ and $ms^2t^6A_{37}$ are crucial in human $tRNA^{Lys}$, which acts as a primer in HIV replication. Modified nucleosides may facilitate or hinder across the loop interactions. Large substituents in 34^{th} and 37^{th} modified nucleosides if oriented suitably may also interact with each other. Across the loop interactions may lead to unconventional anticodon loop structures also affecting flexibility of the anticodon loop. This may restrict or enlarge synonymous codon choice and decoding during protein biosynthesis. Except for $tRNA^{Asn}$ (with interacting Q_{34} and t^6A_{37}), our studies show conventional 'open' loop structure - free of across the loop interactions, for a number of interesting tRNA anticodon loops with diverse hyper modified nucleosides at both of these locations. Molecular dynamics simulations of hydrated anticodon arm of $tRNA^{Asn}$ show persisting interaction involving the diol group of Q_{34} and carbonyl group of ureido linkage in t^6A_{37}. Additionally, the Hoogsteen edge of 37^{th} adenine base participates in hydrogen bonding with Watson - Crick edge of 33^{rd} base and thus contributes to unique loop structure of $tRNA^{Asn}$. Resulting suboptimal Q:C base pairing leads to unbiased reading of U or C as the third codon letter. Absence of queuosine modification, Q_{34}, happens to be also associated with uncontrolled rapid proliferation of cells and malignant growth.

P-993

Structural Studies on Domain 5 of a Group II Intron Ribozyme by Fluorescence Spectroscopy

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Group II intron ribozymes catalyze selfsplicing in bacterial genomes as well as in organellar genes of lower eucaryotes. For correct structure and function these ribozymes need specific concentrations of monovalent and divalent cations such as K^+ and Mg^{2+}. Most of these ions are used for charge screening, but some are also bound to distinct sites fulfilling various specific tasks.
The conserved secondary structure of group II intron ribozymes consists of six domains grouped around a central wheel. Here the focus is set on Domain 5 (D5) of the yeast mitochondrial intron ai5γ, a hairpin of 34 nucleotides, which is crucial for catalytic activity. The 3-nucleotide bulge in D5 is known to be flexible and acts as a metal ion binding platform. We have investigated the binding of different metal ions (Mg^{2+}, Ca^{2+}, Mn^{2+}, Cd^{2+}) to this platform by fluorescence spectroscopy. For this the bulge site adenosine in D5 was replaced by the fluorescent nucleotide base analogue 2-aminopurine (2AP). The binding data fits to an equation describing a binding to a single class of sites. The titration experiments not only reveal different dissociation constants for the tested metal ions but also indicate different effects on the bulge structure.
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Linking of the N-terminus of a peptide to its encoding mRNA

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In evolutionary protein engineering, *in vitro* selection using a cell-free translation system has advantages of large library size and also of applicability to cytotoxic protein.
Although many *in vitro* protein selection techniques such as *in vitro* virus, ribosome display are developed, most of these are the techniques that link the genotype molecule to the peptide at its C-terminus.
We developed a method to link the genotype molecules to the N-terminus of its encoding peptide. The mRNA has DNA-linker hybridize region, translation enhancer, initiation codon, single codon, amber stop codon, N-terminus sequence in GFP gene, His-Tag and ochre stop codon. The mRNA is also linked at 5'-terminus to sup tRNA via spacer and the specific amino acid. The mRNA is translated in the cell-free translation system.
Thus, C-terminus of the protein becomes free. Moreover, in this system, the free protein of the full length is never generated. For this reason, the problem in the conventional technique, that is, the competition between the protein displayed on the genotype molecule and the free protein is eliminated.
We succeeded to turn one round of the " Life cycle " of the *in vitro* virus, judged by His-Tag selection.

P-995

HIV-1 NC-facilitated TAR RNA/DNA annealing is initiated through a loop-loop kissing interaction

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Annealing of the TAR DNA hairpin to a complementary TAR RNA hairpin, resulting in the formation of an extended duplex, is an essential step in the minus-strand transfer process of HIV-1 reverse transcription. In this work, we use gel-mobility-shift analysis to follow the kinetics of this reaction in the absence or presence of HIV-1 NC prepared by solid-phase peptide synthesis. To elucidate the reaction pathway, we use either the complete 59-nt TAR hairpins or truncated 27- to 32-nt minihelices (mini-TAR) derived from the top part (i.e. hairpin loop) of TAR. Assays were also carried out with mutant TAR constructs. The annealing kinetics were studied systematically as a function of DNA concentration and temperature. We show that the annealing initiates through a weak loop-loop kissing interaction, followed by a much slower conversion step, which results in formation of the extended duplex. NC facilitates both reaction steps, resulting in the overall 10^4-fold and 10^6-fold rate enhancement for mini-TAR and TAR annealing, respectively. We show that the kissing step is facilitated by the NC-induced nucleic acid aggregation, which is more pronounced for the longer TAR hairpins. At the same time, the conversion steps in TAR and mini-TAR appear to be very similar and are similarly facilitated by NC 10-100-fold. The later effect relays on the ability of NC to destabilize nucleic acid duplexes, and is equivalent to destabilization of a few base pairs required for the conversion initiation.

P-996

Role of stacking in specific recognition of capped RNA by the CBC protein

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The stacking interaction involving 7-methylguanine moiety of mRNA 5' terminal cap (m^7G) and aromatic amino acid side chains is a common feature of all known cap-binding proteins. The crystal structures of the human Cap Binding Complex (CBC) showed its induced folding upon m^7GpppG cap analogue binding. Stabilization of the CBC-m^7GpppG complex by sandwich stacking of m^7G in between Y20 and Y43 is additionally enhanced by stacking of the second base of capped mRNA with Y138. Gibbs free energy of the association of various CBC mutants with the synthetic cap analogues, m^7GpppG, m^7GpppU, and m^7GTP, has been determined by fluorimetric titration. Preference of the wild type (wt) CBC for the dinucleotide analogues is also observed for the Y43A mutant, with the energy loss of 0.8-1.5 kcal/mol. However, all proteins with the mutated second stacking partner Y20 prefer to bind m^7GTP compared with m^7GpppG. The binding of m^7GTP to the Y20A mutant is only $\sim$0.3 kcal/mol less favourable compared with the wt CBC. These divergences may be ascribed to smaller entropic costs of conformational rearrangement of CBC in the case of a smaller ligand, which can find more favourable contacts when its second part is not efficiently 'constrained' by stacking with Y138.
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Intronic siRNA and miRNA, and DNA methylation

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SiRNA/miRNA can efficiently induce mRNA cleavage or translational repression at the posttranscriptional level in a sequence-specific manner. Recently, it has been shown that these small RNAs guide genome modification in mammalian cells. However, their ability to direct cognate DNA methylation has been confirmed so far only in plants, and their biogenesis, functions, and modes of silencing genes are yet elusive. Here, we report that small RNAs derived from intron regions of some genes can target homologous DNA sequence in promoter, 5'UTR or 3'UTR regions of genes in different human tumor cells. Surprisingly, we also discovered that endogenous siRNAs from introns of genes possessed a large number of target mRNAs by using bioinformatics, and confirmed their existence in human cells with Northern blot analysis. Intronic small RNAs generated by sliceosomes can form mature miRNAs or siRNAs through the processing of Drosha and /or Dicer. RT-PCR analysis indicated that vector-based small RNA repressed expression of homologous genes at the transcriptional and/or translational levels. Western blotting demonstrated that the expression of some proteins was greatly reduced or completely inhibited owing to promoter methylation. These findings reveal that the expression of some genes can incredibly control cell activities at both protein and RNA levels. Our results also suggest that these small RNAs may regulate gene expression in different modes of action.