Posters

– Proton Pumping Systems –

P-1

Binding of V-ATPase inhibitors towards the 4th transmembrane segment of the enzyme´s subunit c

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The indole class of V-ATPase inhibitors (SB 242784 and INH-1 among others) possesses high inhibitory activity towards V-ATPases and exhibits high selectivity for the osteoclast form of the enzyme (Gagliardi et al. 1998). The most promising of these compounds for osteoporosis treatment, **SB 242784**, was shown to be extremely effective in preventing bone-loss in animal studies (Visentin et al. 2000). It is currently believed that the site of action of these inhibitors is likely to be located in the membrane bound c-subunits of V-ATPases. To address the identification of the inhibitor molecular target(s) in the c subunit of V-ATPase, the specific binding of the inhibitors to selected transmembrane segments of c-subunit isoforms containing a proposed binding site for the inhibitor was investigated using fluorescence methodologies. Bafilomycin, a natural and more potent inhibitor of the enzyme was able to bind strongly to the selected transmembrane segment, but binding of SB 242784 and INH-1 was not significant, suggesting that the binding site of the indole class of V-ATPase inhibitors includes residues outside this segment.

P-2

The temperature dependence of the ultrafast electron transfer in cytochrome c oxidase

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Electron transfer (ET) within proteins occurs via chains of redox intermediates that favor directional and efficient electron delivery to an acceptor. Individual ET steps are energetically characterized by the electronic coupling V, driving force ΔG, and reorganization energy λ . λ reflects the nuclear rearrangement of the redox partners and their environment associated with the reactions; $\lambda \approx 700-1100$ meV has been considered as a typical value for intraprotein ET. In non-photosynthetic systems, functional ET is difficult to assess directly. However, using femtosecond flash photolysis of the CO-poised membrane protein cytochrome c oxidase (CcO), the intrinsic rate of the low-ΔG electron injection from heme a into the heme a_3-Cu_B active site was recently established at $(1.4\ \text{ns})^{-1}$. Here we determine the temperature dependence of both, the rate and ΔG of this reaction and establish that this reaction is activationless. Using the quantum mechanical form of non-adiabatic ET theory and common assumptions for the coupled vibrational modes, we deduce that λ<200 meV. It is demonstrated that the previously accepted value of 760 meV actually originates from the temperature dependence of Cu_B-CO bond breaking. We dicuss that low ΔG, low λ reactions are common for efficiently channelling electrons through chains buried inside membrane proteins.

P-3

Action potential in Chara cells affects the thylakoid pH gradient and photosynthetic electron flow

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The propagation of action potential (AP) in *Chara corallina* led to a transient suppression of all pH bands in illuminated cells. The AP caused a large pH decrease in the basic zones and a slight pH increase in the acid zones. Clearly, membrane excitation interferes with light-dependent mechanisms of pH banding. In resting *Chara* cells the pH pattern correlated with the quantum yield of photosystem II electron flow (ΔF/Fm'). The AP caused a fast transient decrease of ΔF/Fm' and Fm' fluorescence in the basic cell regions but had little effect in the acid regions. The decrease of ΔF/Fm' in the basic region suggests that linear electron flow is retarded after AP. In addition, rapid AP-induced drop of Fm' is indicative of energy-dependent nonphotochemical quenching (NPQ) associated with ΔpH at the thylakoid membrane (TM). The mechanism of AP-induced increase of ΔpH may involve the increase in cytosolic Ca^{2+} during excitation. This should transiently elevate the Ca^{2+} level in the chloroplast stroma, inhibit the Calvin cycle, and lead to over-energization of TM (the increase in ΔpH and NPQ). The subsequent recovery of Fm' might be due to the operation of the Ca^{2+}/H^{+} antiporter of TM, which should reduce the stromal Ca^{2+} level and ΔpH. Thus, the electrical excitation of the plasma membrane initiates in the basic cell region a pathway that can modulate membrane events in thylakoids.

P-4

Redox-linked protonation changes in cytochrome bc1 identified by Poisson-Boltzmann electrostatics

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Cytochrome bc1 is a ubiquitous protein in cellular energy conversion. It is a transmembrane protein complex that transfers electrons from reduced coenzyme Q to cytochrome c, and translocates protons across the membrane. To evaluate conflicting mechanistic models, it is necessary to identify protein residues that are responsible for the redox-linked (de)protonation reactions that are part of the catalytic cycle.
Poisson-Boltzmann electrostatics are a valuable tool to characterise protonation behaviour in large systems. The results can be compared to FTIR experiments and then extend and aid in the interpretation of these data. Calculations have been performed based on the crystal structures of cytochrome bc1 from yeast. A CoQ molecule has been modeled into the Qo active site. Two different conformations of the Qo site as revealed by the crystal structures have been considered.
Protonation probabilities of all titratable groups in the protein have been calculated, once for the completely oxidised system, and once for the completely reduced system. Only few residues change their titration behaviour upon reduction of the redox-active cofactors of cytochrome bc1. These residues are candidates for mechanistically relevant (de)protonation reactions. The obtained results are discussed with respect to the mechanistic details of proton and electron transfer in cytochrome bc1.

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P-5

Allosteric regulation of the Na^+/H^+ exchanger NHE-1 by cell membrane shape and composition

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NHE-1 is an ubiquitously-expressed electroneutral mammalian Na^+/H^+ exchanger which maintains cytosolic pH and plays key roles in others cellular functions such as volume regulation, migration, proliferation and apoptosis. Its activation by intracellular acidification exhibits a cooperative behaviour for protons which increases upon growth factor stimulation. We have recently shown that this phenomenon can be described by a Monod-Wyman-Changeux model. In this mechanism, NHE-1 which is under a dimeric form, oscillates between a low and a high affinity conformation. Under resting conditions, the major form of NHE-1 is the low affinity exchanger. When the cytoplasm becomes acidic, the exchanger is converted into the high affinity form which actively extrudes protons from the cytoplasm. This new model was used for the quantitative characterization of NHE-1 response to different changes in membrane environment. The effects of membrane shape, tension and lipid composition on this allosteric transition will be presented. Implications for cell volume regulation will be discussed, in the light of functional sites possibly involved in this mechanism.

P-6

Rhodothermus marinus complex I operons contain a NhaD Na^+/H^+ antiporter and pcd homologues

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Rhodothermus marinus is a microaerophilic bacterium from the *Flexibacter*, *Bacteroids* and *Cytophaga* group that grows optimally at 65°C and 2% NaCl. The respiratory chain of this organism contains a rotenone-sensitive NADH:menaquinone oxidoreductase (Nqo) that catalyses the oxidation of NADH, reducing menaquinone. Concomitantly with this process protons are translocated across the cytoplasmic membrane. The purified enzyme has a non-convalently bound FMN and showed at least 14 subunits, by SDS-PAGE, and the presence of at least five iron-sulphur centres: two $[2Fe\text{-}2S]^{2+/1+}$ and three $[4Fe\text{-}4S]^{2+/1+}$ centres by EPR spectroscopy [1]. In this work, genetic evidence for the presence of *R. marinus* type I NADH:menaquinone oxidoreductase was provided. In fact, two operons encoding for this enzyme were revealed: nqo_A and nqo_B, comprising the *nqo1 to nqo7* and *nqo10* to *nqo14* genes, respectively. Furthermore, *nqo8* and *nqo9* genes were found in a different part of the genome. Two unexpected genes, encoding for a homologue of a NhaD Na^+/H^+ antiporter and a pterin-4α-carbinolamine dehydratase (PCD) were also found within nqo_B and shown to be co-transcribded with *nqo13* and *nqo14*. Southern-blot analysis with *R. marinus* genomic DNA probed with *nqo1* and *nqo12* showed that there is one single copy of these two genes in *R. marinus* genome.

[1] Fernandes *et al* (2002). *J. Bioenerg. Biomembr.* 34(6): 413-21

P-7

Essential Arg of a subunit in FoF1-ATP synthase plays a key role in c-ring rotation

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In a rotary motor F_oF_1-ATP synthase (F_oF_1), the ring of F_oc subunits (*c*-ring) rotates relative to F_oa subunit as protons flow through channel(s) in F_oa and a carboxyl group in F_oc. Previous reports have indicated that a conserved Arg in F_oa controls the proton transfer at the F_oa/*c*-ring interface. In fact, thermophilic F_oF_1 with substitution of this Arg (*a*R169) to Glu, Ala, Val, Ile, Lys, Phe, or Trp lost proton-coupled ATP hydrolysis/synthesis activities, that is, no rotation occurred in these mutants. However, the mutants *a*R169E, *a*R169A and *a*R169V, but not other mutants, still mediated passive proton translocation. This proton translocation was completely blocked by the second mutation (*c*E56Q) of F_oc. Then we generated a 'rotation-impossible' (c_{10}-*a*)F_oF_1 in which ten copies of F_oc in the *c*-ring and F_oa were all genetically fused as a single polypeptide. This (c_{10}-*a*)F_oF_1 had a native-like structure because activities appeared upon cleavage of c_{10}/*a* linkage. We found that (c_{10}-*a*)F_oF_1 did not mediate passive proton translocation but (c_{10}-*a*)F_oF_1 with *a*R169A mutation did so though slowly. Thus, it appears that the large, delocalized positively-charged side chain of the conserved Arg in F_oa ensures proton-coupled *c*-ring rotation by preventing futile proton shortcut.

P-8

The Yolk granules dynamics during Periplaneta americana embryogenesis

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In insects the embryo development occurs totally disconnected from maternal organism, so the presence of all the nutritive reserve (like proteins and lipids) inside the egg is indispensable for embryo development. The Yolk granules (YG) occupy almost all of the egg extension. However the yolk is not available to the embryo while it is inside the granules. These organelle`s acidification is essential for yolk liberation by destabilization of YG`s structure. In this work we intend to describe differential acidification and fusion mechanisms of YGs during early *P. Americana* embryogenesis. Oothecae`s contents were extracted and submitted to acridine orange uptake for monitoring YG`s acidification by fluorescence confocal microscopy. The eggs were also submitted to preparation for scanning and transmission electron microscopy. During the first day of embryogenesis we can observe differential small YGs acidification after PPi addition. During the fifth day, we can notice structures were small YG are associated with big YGs, apparently promoting their acidification. The YG fusion is showed by scanning and transmission microscopy in eggs from fifth day of embryogenesis. Since the egg is formed with all yolk necessary for entire embryo development, it seems necessary some kind of regulation upon how and when the yolk will be liberated from inside the granule structure. Differential YG`s acidification and fusion can be a well regulated process for controlling yolk liberation during the insect embryogenesis.

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– Proton Pumping Systems –

P-9

A H+PPase on yolk granules acidification during early P. americana embryogenesis

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The H+PPases catalyzes both the hydrolysis of PPi and the eletrogenic translocation of protons into cell compartments. In insects the yolk proteins are incorporated by the oocyte during the oogenesis and are accumulated in structures called yolk granules (YG). Its acidification is essential for activation of acidic enzymes responsible for yolk liberation during embryo development. This work has described and localized a H+PPase pump associated to membrane fractions of small vesicles present inside the eggs of the insect *Periplaneta americana*. The enzyme activity was tested on preparations of membrane fractions from *P. americana* eggs. The YG were extracted from the ootheca and submitted to acridine orange uptake in fluorescence confocal microscopy. The YG were also submitted to citochemistry incubation followed by preparation for transmission electron microscopy**.** The enzyme activity showed total dependence on Mg2+ and was strongly inhibited by NaF, Ca2+ and IDP, maintaining the kinetics properties previously described. We showed differential acidification where small YG are, preferentially, acidified after PPi addition, supposing the existence of H+PPase pump in this mechanism. After citochemistry assay the precipitates formed by the enzyme product complexed with Fe2+ is clearly showed along the small YG`s membrane, in accordance to the preview result. These data together can affirm the presence of a functional H+PPase participating on YG acidification during early embryogenesis of the insect *P. americana*.

P-10

The kinetics of the photochemical reaction cycle of ion transporting retinal proteins

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The kinetics of the photochemical reaction cycle of the bacteriorhodopsin, pharaonis halorhodopsin and proteorhodopsin were determined in H_2O and D_2O at low and high pH, to get insight in the proton dependent steps of the transport process. While all the steps of the bacteriorhodopsin photocycle at normal pH exhibited a strong isotope effect, the proton uptake step of the photocycle, measured at high pH, became independent of deuterium exchange, making plausible that this step, at low proton concentration, becomes concentration dependent, not mobility dependent. The proton transporting photocycle of the proteorhodopsin at its normal pH (9.5) shows a marked deuterium effect, while at high pH (12.2) this effect almost totally disappears. In the case of halorhodopsin in all the chloride, nitrate and proton transporting conditions the photocycle was not strongly affected by the deuterium exchange. While in the cases of the first two ions this seems normal, the absence of the deuterium effect in the case of the proton transporting photocycle was a puzzle. The only plausible explanation is that in the presence of azide the halorhodopsin transports not the proton, but a negatively charged ion the OH^-, the mass and mobility of which is only slightly influenced by the deuterium exchange.

P-11

A look at the bacterial proton ATPase under fermentation: properties, energetics and regulation

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The findings for *Escherichia coli* are prominent for the functioning proton F_0F_1-ATP synthase under fermentation (see [1]): an ATP-dependent H^+ pumping is different under anaerobic and aerobic conditions; the expression of the genes coding subunits of F_0 responds to anaerobic conditions; under fermentation the F_0F_1 content in the membrane is lowered.
The study of this complex under fermentative growth have led to hypothesis [1] that, in the absence of oxidative phosphorylation, F_0F_1 is implicated as an essential part of H^+ movement and ATP hydrolysis, associated with secondary transporters (K^+ uptake system) and/or anaerobic redox enzymes (formate hydrogenlyase). These associations can result from a protein-protein interaction by dithiol-disulfide interchange, and the latter is a way to transfer energy from F_0F_1 to the other protein; reducing equivalents are required. The energy released ($\sim$33 kJ/mol) could be sufficient for useful work (K^+ uptake). The change in accessible SH-groups number defined with membrane vesicles (*N*-ethylmaleimide (NEM)-sensitive increase by ATP that is inhibited with *N,N'*-dicyclohexylcarbodiimide and azide and absent in *atp* mutant) is an argument in favor to a dithiol-disulfide interchange. The effects of oxidizers (ferricianide) and reducers (DL-dithiothreitol, Cu^{2+}) studied suggest a role of redox potential in regulation of F_0F_1.
Reference: [1] A. Trchounian, Biochem. Biophys. Res. Commun. 315 (2004) 1051-1057.

P-12

A new theoretical approach to study proton channels in the Quinol Oxidase aa3 from A. ambivalens

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The Quinol Oxidase from *A. ambivalens* is a membrane bound protein that belongs to the family of haem-copper oxidases. This kind of proteins catalyses the reduction of oxygen to water in a process coupled with the translocation of protons across the mitochondrial or cytoplasmic membrane. Given that the catalytic activity of this kind of proteins occurs well inside the protein matrix (haem-copper centre) in their subunit I, proton channels must exist, not only to allow the described proton pumping mechanism but also to allow the delivery of protons necessary to catalyse the reduction of oxygen to water. Due to the structural divergence found in the protein superfamily, several different channels have been previously suggested based on structural and mutagenic data. The Quinol Oxidase is an example of this kind of proteins where the topological distribution of the residues in described proton channels departs from that normally found in haem-copper oxidases. In order to understand how the proton pumping mechanism occurs in this protein, we have combined molecular modelling techniques with a statistical analysis of the distribution of internal water molecules. From our results we were able to suggest two spatial homologous proton channels, when compared to the canonical oxidase from *P. denitrificans,* and a third channel with a unique structural position among all terminal oxidases with known structure.

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– Functional Cell Imaging –

P-13

Strong Cells get the Beat — Forces of Cardiac Myocytes

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Generating forces is the most important physiological function of heart muscle cells. Here we present measurements of the forces of single myocytes. Cells from rat embryos are cultivated on elastomeric substrates consisting of a 100 μm thick film of biocompatible crosslinked poly-dimethyl-siloxane (PDMS, Young module of $\sim$30 kPa) on cover slides covered with proteins of the extracellular matrix. The surface of the elastomer carries a regular pattern of microdots. Force production of the cells results in a deformation field of the elastomer which is measured via observation of the microstructures on the interface between elastomer and cell culture medium. Cells and surface deformations are observed for longer periods by live cell imaging. Focal adhesions are localized by Reflection Interference Contrast Microscopy. Subsequently, deformation fields are extracted by digital image processing. From these fields cell forces are calculated by the method of Balaban et al. (Nature Cell Biol. 3 pp 466 (2001)). We present detailed results on the magnitude, the spatial distribution and the time course of forces of beating cells. Moreover, the correlation between force development and Ca^{2+} influx during individual contractions is studied.

P-14

Cofilin, actin and their complex observed *in vivo* using fluorescence resonance energy transfer

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Actin is the principal component of microfilaments. Their assembly/disassembly is essential for cell motility, cytokinesis and a range of other functions. Recent evidence suggests that actin is present in the nucleus where it may be involved in the regulation of gene expression. Cofilin binds actin and can translocate into the nucleus during times of stress. In this report, we combine fluorescence resonance energy transfer (FRET) and confocal microscopy to analyse the interactions of cofilin and G-actin within the nucleus and cytoplasm. By measuring the rate of photobleaching of fluorescein-labeled actin in the presence and absence of Cy5-labeled cofilin, we determined that almost all G-actin in the nucleus is bound to cofilin, whereas approximately half is bound in the cytoplasm. While imaging FRET we observed that a significant proportion of fluorescein-labeled cofilin in both the nucleus and cytoplasm binds added TMR-labeled G-actin. Our data suggests there is significantly more cofilin-G-actin complex and less free cofilin in the nucleus.

P-15

High rate stimuli preferentially release dense core vesicles close to membrane in autonomic ganglia

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We are interested in studying the dynamics of the large dense core vesicles (LDCV) in response to high frequency stimulation. We addressed this issue analyzing synaptic boutons ultrastructure by electron microscopy; we studied the number of LDCVs and their relationship with plasma membrane and active zones, in boutons of control and stimulated ganglia. We found an average of 5.3$\pm$0.5 LDCV/bouton, 60% of the vesicles were at <=100 nm of the plasma membrane, regardless the distance to the active zone. Stimulation train (40Hz, 1 min) decreased significantly the number of LDCV to 3.8$\pm$0.3 LDCV/bouton (P=0.008), the LDCV at <=100 nm of the membrane decreased from 3.1$\pm$0.2 to 2.1$\pm$0.1 LDCV/bouton (P=0.003); while the change of LDCV at > 100 nm from the plasma membrane was not significant. Regarding the relationship with the active zone, LDCVs locate either at <= 100 nm or at 500-600 nm away showed a significant reduction (0.4$\pm$0.1 vs. 0.2$\pm$0.1 and 0.6$\pm$0.1 vs. 0.4$\pm$0.1 LDCV/bouton; P=0.03). The histogram of dense core optical density showed a negative skewness, indicating a larger proportion of less dense vesicles; the stimulated group showed a trend to increase this population of vesicles. Our data suggest that LDCV located at <=100 nm to the membrane present a high probability to be released; while optical density analysis imply that LDCV exocytosis can be either partially or totally. (DGAPA IN217702)

P-17

Diffusion of eqFP611 molecules in living interphase HeLa cell nuclei studied with FCS

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Diffusion of the far-red fluorescent protein eqFP611 was studied in HeLa cell nuclei tagged with fluorescent GFP-H2B histons. The cells were used transformed with a GFP-H2B fusion protein as a chromatin marker and with freely diffusing eqFP611 protein, and observed using Fluorescence Correlation Spectroscopy (FCS) with a scanning two-color confocal detection system. From the H2B-GFP fluorescence the chromatin density in the cell nucleus was quantified.
With this set-up, we investigated whether there is a correlation between the chromatin density and the mobility of eqFP611 molecules. In an extensive set of measurements we could show that the mobility of eqFP611 does not depend on the chromatin density, i.e., all of the intranuclear space is freely accessible to small proteins of the size of eqFP611 (about 26 kDa) without regards to the chromatin density.

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– Functional Cell Imaging –

P-18

Live cell fluorescence lifetime spectro-imaging applied to the interactions of a cardiac K+ channel

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Fluorescence lifetime imaging is a promising technique for monitoring the specific interactions between proteins by Fluorescence Resonance Energy Transfer (FRET). In this technique, the variation of the donor lifetime in the presence of the acceptor is the imaged parameter. We have performed a detailed study of the different factors influencing the measured fluorescence decays of wtGFP and CFP under (i) two-photon TCSPC microscopy and (ii) one-photon time-gated Nikon FLIM imaging. Factors of variability such as the excitation regime, the cell physiological state, the temperature or the nature and local concentration of the fluorescent construct have been studied. Different control experiments for FRET quantification are compared by use of CFP-YFP chimaeras. Provided a proper control of a few crucial parameters, highly accurate and reproducible values of the intracellular CFP average lifetime can be obtained, allowing the quantification of average FRET efficiency at percent accuracy with minimal inter-cellular variability. These results are used to monitor, by methods (i) and (ii) above, the subunit interactions of a cardiac potassium channel Kv1.5 expressed in CHO cell lines.

P-19

Using simple nervous system to cope with a more complicated one

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The central nervous system (CNS) of highest vertebrates is a hard system for analysis and it is often more effective to study simple nervous systems of invertebrates. This maneuver allows performing analysis of CNS function by the wide range of experimental approaches: from behavioral to cellular and molecular. CNS of a medicinal leech is a convenient model for such a complex researches. Our interests are focused to combine and compare to each other the experimental data for isolated leech neurons and semiintact preparations, obtained by electrophysiological technique, fluorescent and interference microscopy. The interconnections between intrinsic optical properties, electrophysiological responses, calcium redistribution and mitochondrial metabolism during natural neuronal activity and artificial stimulation were revealed. Inhibitory analysis was performed to elucidate the mechanisms of intracellular processes interplay.

P-20

Dynamics of nanocontractions in cardiomyocyte visualized with harmonic generation microscopy

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Functional investigations of isolated cardiomyocytes have been performed with a simultaneous detection second harmonic generation (SHG), third harmonic generation (THG) and multiphoton excitation fluorescence (MPF) microscope. The SHG imaging revealed nanocontractions of sarcomeres on the time frame of a few hundred milliseconds. The nanocontractions of sarcomeres were not synchronized and did not produce macrocontractions of the whole cell. THG highlighted mitochondria and showed the intensity variations "flickering" similar to the once previously observed with MPF. The flickering was sensitive to uncouplers and inhibitors of electron transport. Flickering of mitochondria is more easily observable in THG images than in fluorescence where the flickering is superimposed on a monotonous decrease of fluorescence due to photobleaching. Structural crosscorrelation analysis of the images obtained with the different contrast mechanisms rendered a colocalized image where correlated and anticorrelated structures were positioned in the 3D-image. The simultaneous detection SHG, THG and multiphoton excitation fluorescence microscope proved to be a very powerful tool for investigating interactions of different organelles inside a cell.

P-20-B

Investigating structure and kinetics of triple-labeled biomolecules

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Single- and double-labeled molecules are well-established systems for a variety of fluorescence applications, thus helping to characterize bimolecular reactions in vitro and in vivo and intracellular spatial localization. Especially in more complicated biological reactions, however, generally more than two molecules are involved. Elucidation of such mechanisms requires not only sequential pair wise labeling of the potential reaction candidates, but also repeated measurements under comparable conditions.
One possibility of alleviating this problem consists in extending conventional fluorescence techniques, e.g. FRET or FCS, to the recordings from three spectrally distinct chromophores. Whereas especially FCCS (Fluctuation Cross-Correlation Spectroscopy) gives unequivocal information about correlated movements of the respective particles, FRET also yields valuable clues about the spatial proximity of the different chromophores.
Here we show examples for the combination of FCCS with triple-FRET (triFRET), which allows monitoring a three-component system with a single excitation wavelength. First proof of principle measurements were performed on a double stranded DNA model system with well-known geometrical properties. More advanced applications focus especially on ternary complexes to unravel information about interactions of more complex biologically relevant systems.

Posters

– Functional Cell Imaging –

P-21

Force evaluation of adherent myocytes

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Almost all cells are able to create mechanical forces depending on the cytoskeleton and focal contacts linking the cytoskeleton to extracellular structures. A measuring technique must possess high spatial resolution to discern the contribution of those foci and must be able to resolve forces in the nN range. We have established microstructured ultrasoft substrates for measuring forces of rat heart muscle cells. These cells are comprised of actin-myosin fibers with a regular orientation to each other. Forces created by actin-myosin contraction deform the substrates primarily at the fibers' ends. Subsequently deformations can be mapped by video microscopy and analyzed using digital image processing and elasticity theory to reconstruct the forces at focal contacts (Balaban et al. Nat Cell Biol. 2001). Since focal contacts of actin-myosin fibers often end in some distance to the cell edge, small deformations are hardly to detect using standard microscopy techniques. Reflection interference contrast microscopy allowed us to visualize the whole elastomeric surface under the cell and therefore to detect any surface deformation created by the cell. In addition, the experiments showed that a myocyte does not only adhere to an elastomeric surface at the ends of actin myosin fibers. Instead, myocytes are connected to the substrate at every sarcomeric subunit of these fibers resulting in a force application at many different sites of the same contractile fiber to the surface.

P-22

A novel 8-dimensional microscope and its applications in investigation of Instant effects of external stimuli on living cells

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A novel 8-dimensional microscope is developed to perform simultaneous measurement on the structure and function of single intact cell. The technical system is based on an inverted fluorescent microscope, its information acquisition and processing is organized in 8-dimensions: 3-D image is acquired by a 3 dimensional relief image construction system; the 4^{th} dimension(wave length) is taken by a fast micro-spectrophotometry; which, accompanied with a dynamic image analyzing system, also gives the information in the 5^{th} dimension (time) by giving the absorption spectra and images in a time-lapse mode; the 6^{th}–8^{th} dimensions are organized by the lights from the sample: the transmitted light, gives the information of image and absorption spectroscopy; the scattered light, gives the information about the dynamic parameters of intracellular molecule and the cell membrane; the fluorescence, gives the information of the labeled molecules in the cell. Therefore, the 8-dimensional microscope is capable of performing measurement on the parameters of single intact cells include: 1) the chemical structure and concentration as well as the hydrodynamic radius of the intracellular molecules; 2) the 3-D morphology of the cell; 3) the functional change of the intracellular protein; 4) the flexibility and rigidity of the cell membrane; 5) the differentiation, proliferation and the activity of the cell. The instant effects of temperature and 900MHz electromagnetic radiation on human RBC investigated using the technique were also reported.

P-23

Secretory vesicle motions studied by total internal reflection fluorescence microscopy

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Total internal reflection fluorescence microscopy (TIRFM) is well adapted to the study of the motions of secretory vesicles located in the vicinity of the plasma membrane. Using this technique, we have monitored trajectories of fluorescently labeled subplasmalemmal vesicles in 3 dimensions.
In order to better understand the interactions of the vesicles with their complex environment (cytoskeleton, membrane), we applied two different approaches: (i) we developed a software to dissect complex vesicle trajectories, (ii) we modified the microscope for dual-color observation in live cells.
In approach (i), a given trajectory is divided into sub-trajectories, each exhibiting a distinct behavior (random, constrained or directed motion). These sub-trajectories are then quantitatively analyzed. This method allowed us to characterize precisely dynamics of subplasmalemmal vesicles.
Using approach (ii), we directly visualized interactions between vesicles and cytoskeleton. Firstly, simultaneous labeling of microtubules and vesicles showed that a small fraction of vesicles move along microtubule tracks rapidly ($\approx 1\mu$m/s) and over long distances ($> 5\mu$m). Secondly, simultaneous labeling of vesicles and actin filaments revealed that some vesicles are clearly attached to actin filaments.

P-24

Imaging of electrical dynamics in cultured brain slices by multi-transistor-array (MTA) recording

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Direct electrical interfacing of semiconductor chips with neuronal tissue may lead to novel experimental approaches in brain research and also give rise to hybrid computational devices. Here we report on a time-resolved imaging of the electrical activity in organotypic brain slices from rat hippocampus by multi-transistor-array (MTA) recording on an area of 1 mm^2 at a resolution of 7.8 μm and 0.5 ms. Brain slices were cultured on the inert titanium dioxide surface of silicon chips fabricated by an extended CMOS process. Upon stimulation in the CA3 region we observed fast propagating waves of negative field potentials which we assign to orthodromic and antidromic action potentials in the mossy fibers and slower transient field potentials of postsynaptic activity in CA3 and CA1 with negative sign in stratum radiatum and positive sign in stratum pyramidale. The transistor signals matched local micropipette recordings of electrical field potentials in amplitude and shape. Direct interfacing of an MTA chip provides a complete observation of neuronal signaling in an extended area of brain tissue. This technique is suitable to elucidate the functionality of planar neuronal systems at a high resolution.

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– Functional Cell Imaging –

P-25

Force-induced biological modifications in *in vitro* cultured epithelial cells

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Characterizing the mechanical behavior of eucaryotic cells is essential to understand biological functions like cell division or migration. Here we measure a stress/deformation relationship under constant or oscillatory excitation in epithelial cells.
We developed an Optical Tweezers set up allowing to apply controlled forces on micrometric beads coated with a protein specifically recognized by integrins or other types of membrane receptors, and anchored to the actin cytoskeleton *via* this receptor adhesion complex. While exerting a force on the bead, we follow the repartition of fluorescent proteins involved in the cell dynamic response. After a few minutes, actin is recruited around the anchoring site, increasingly with the trap rigidity. By applying a sinusoidal deformation to the cell at different frequencies, we retrieved the storage modulus G' and loss modulus G" for various cell types (epithelial, myoblasts, fibroblasts, macrophages)[1]. The variation of G' and G" for each tested cell appears to conform to a power law of frequency ($G' \propto f^{\alpha}$; $G'' \propto f^{\beta}$; with $<\alpha> \cong <\beta> \cong 0.2 \pm 0.05$). This law is very robust since it is independent on the cell type and on the bead-cell anchoring type. We currently measure a local creep function using the same technique, by keeping the trapping force constant, in order to compare these measurements to the creep function obtained by fully stretching cells with a microplates technique [2].
[1] Balland *et al*. Eur. Biophys. J., *in press* (2005).
[2] Desprat *et al*. Biophys. J., 88: 2224-2233 (2005).

P-26

Sensitivity of CFP fluorescence to the physico-chemical environment: implications for cell imaging

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One of the most widely used donor for intensity-based and FLIM-FRET experiments is the cyan (CFP) variant of the Green Fluorescent Protein *Aequoria Victoria*. It was commonly proposed that the bimodal feature of the absorption and emission spectra as well as the biexponential nature of the fluorescence decays of CFP originate from the two conformations detected crystallographically or by ^{19}F NMR. In the present work, we show that a more complicated model is required to fully describe the photophysical behavior of CFP. We also point to the strong dependance of the fluorescence properties of CFP on temperature and pH which have substantial consequences for intensity and lifetime imaging. Furthermore, a concentration-dependent variation of the CFP lifetime either in the absence or in the presence of acceptor have been observed in living cells. This phenomenon probably originates from intermolecular energy transfer and is expected to affect the FLIM-FRET as well as the intensity-based FRET imaging. The present work contributes to a better knowledge and interpretation of the fluorescence signals of CFP obtained in living cell imaging.

P-27

Regularization of a method of a scattering of laser radiance

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Definition of properties of material by a scattering of radiance is the important task with which connected series of biophysical problems [1,2]. This work is devoted a methodical questions application of multipurpose analytical system on test objects. Cell volume is important parameter of a function and structural state of cell [3]. The regularizing method of scattering of laser radiance allows to receive a size distribution functions of cells and to trace their time evolution with the high time resolution.Volume received by a regularizing method of a lazer light-scattering was compared with actual volume of cells. The calibration curves were constructed on the basis of it. Influence of change of the cell shape was tested also on results of gauging of on volume of erythrocytes. Results obtained in the given work show, that the regularizing method is sensitive to change of volume of cells. 1. Shifrin.K.S. Vvedenie v optiku okeana.L.: Gidrometeoizdat. 1983. 2. Van de Khjulst. Rassejanie sveta malymi chasticami. M: Inostrannaja literatura. 1961. 3. 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-28

Data analysis methods for studying the role of calcium binding proteins in axonal Ca^{2+} dynamic

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This work has a double aim : 1) a better understanding of the role of calcium binding proteins in axonal calcium dynamics in mouse cerebellar neurons, 2) the adaptation of recent statistical methods to the analysis of calcium imaging experiments.
Among intracellular messengers, calcium ions play a central role. They integrate and coordinate numerous cellular events. In neurons, they trigger neurotransmitter release and are involved in synaptic plasticity. Cells are therefore cautiously regulating their cytosolic calcium concentration through an interplay between calcium channels, intracellular stores and various calcium binding proteins.
Not surprisingly, calcium homeostasis disorders have been implicated in various diseases like Alzheimer's, Parkinson's disease or trisomy 21. In such a context, a better understanding of the role of calcium binding proteins could lead to better diagnostic and/or treatment methods.
We propose an approach where multiple competing models of intracellular calcium dynamics combined with a model of data collection (2-photons or CCD fluorescence calcium imaging, linked to electrophysiological recordings) are fitted and compared. Individual models are fitted using the maximum likelihood procedure (leading to confidence intervals). Model comparison is done with the Akaike Information Criterion (AIC). The wide applicability and sound theoretical foundations of these methods make them very attractive to people involved in data analysis problems.

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– Functional Cell Imaging –

P-29

Protection capability of polyelectrolyte layers: a Saccharomyces/Paramecium model

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The development of an encapsulation method for biological cells can open new avenues in the realization of artificial cellular networks and smart biosensors. The capsular material should be restrictive enough to prevent encapsulated cells from the attack of specific proteins and enzymes filtered by the permeability properties of the capsule itself. A suitable system to this end can be designed and realized using polyelectrolytes assembled by means of the Layer-by-Layer-technique. A feature of the capsules is the possibility to control their properties on a nanometer scale for tuning the wall texture by optimizing the preparation conditions. In the present work we assess the ability of polyelectrolyte nanostructured shells to protect *Saccharomyces cerevisae* yeast cells inserted within a heterologous organism, the protozoan *Paramecium primaurelia*, against lysosomial enzymes attack. To investigate the influence of preparation conditions we used PE solutions at different ionic strengths. As well, we increased the number of layers of the shell in order to derive those conditions enabling protection of the cells from digestion by *Paramecium*'s lysosomal enzymes. Yeast cells viability was tested by observing population growth dynamics. This study has been carried out by means of confocal laser scanning microscopy and fluorescence labelling.

P-30

Ligand-induced monomer/dimer dynamics of the urokinase receptor in live cells by 2P-FLIM/FCS

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The urokinase receptor (uPAR) is a multifunctional receptor, which regulates cell adhesion, migration and proliferation in normal and pathological situations. It is currently unknown how the GPI-anchored uPAR transmits a variety of signals into the cell.
We follow the effect of uPAR ligands on live HEK293 cells expressing functional fluorescent chimeras of uPAR, in which EGFP or spectral variants, were inserted between the GPI-anchor and the D3 receptor domain. By combining 2-photon FLIM and fluorescence fluctuation spectroscopy (FCS, PCH), we show that uPAR monomers and dimers are heterogeneously distributed in membrane microdomains. Dimers increase in the cell-to-cell contacts as well as in the presence of the amino terminal fragment of the ligand uPA. In contrast, PAI-1-dependent internalization of uPAR results in a reduction of uPAR dimers exposed in the plasma membrane. These results support the hypothesis that uPAR monomer/dimer interplay can be regulated by extracellular ligands such as uPA and PAI-1, introducing a novel concept that might contribute to understand the function of the receptor in signaling.

P-31

Living cytoskeleton rheology: comparison to semi-flexible polymer models

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Understanding the viscoelastic properties of the cytoskeleton is a necessary step to go further in the comprehension of numerous biological processes such as cell migration or cell division, in which the mechanical behavior of the cytoskeleton plays a crucial role. By extending the frequency range of magnetic twisting cytometry and applying a new non-invasive method, two-point microrheology, we probe the mechanical response of several types of cultured cells over a wide frequency range. The dynamic shear moduli of both have a $\omega^{3/4}$ behavior at high frequencies and similar anomalous length-scale dependence at intermediate frequencies. Moreover, the F-actin elastic plateau at low frequencies is replaced by a weak power-law frequency dependence in the cell case in agreement with previous experiments. This observation has been interpreted as evidence for the SGR model, a trap-type glass model with an effective temperature. In contradiction, we show, using, drugs, that the power-law behavior is ATP-independent and that the mode of relaxation should thus be thermally activated.We propose a simple, alternative model of cytoskeletal mechanics based on the thermally activated, forced unfolding of domains in proteins cross-linking a stressed semi-flexible polymer gel. It directly relates a cell's mechanical response to biophysical parameters of the cytoskeleton's molecular constituents. The model suggests natural explanations for the observed correlation between cell rheology and intracellular static stress.

P-32

Cellular stiffness response to sequential external deformations in a single fibroblast

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Stiffness responses of living fibroblasts were measured by scanning probe microscopy, following elongation or compression by deformation of an elastic substrate by 8%. The cellular stiffness, reflecting intracellular tension acting along stress fibers, decreased or increased instantly in response to the elongating or compressing stimuli, respectively. After the rapid change of stiffness, the fibroblasts gradually recovered to the initial stiffness within the following 2 hours, and then tended to be constant. The cells did not show conspicuous changes in shape after the 8% deformation during the SPM measurements. These results indicate that fibroblasts have a mechanism that regulates intracellular tension along stress fibers to maintain the cellular stiffness in a constant equilibrium state. Furthermore, when the cell undergoes various patterns of sequential deformations, its cellular stiffness response will be reported.

Posters

– Functional Cell Imaging –

P-33

Antistatic phase plates opening supramolecular biology with electron microsocpy

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Due to the severe obstacle of the charging, phase contrast methods using phase plates have not yet been materialized. We have completely solved the phase-plate charging problem and invented two kinds of phase contrast transmission electron microscopy; Zernike phase contrast (ZPC) and Hilbert difference contrast (HDC). They have been applied to unstained ice-embedded samples such as proteins, viruses, whole cells and tissue sections with a great success of contrast enhancement. In this report, particular focus is given to the antistatic technology, which has long been awaited to enable the kind of phase contrast using phase plates, and to the quantitative comparison of frequency dependent contrasts between the conventional and the innovated to clarify what is actually improved with phase plates.

P-34

Cell surface localisation of Kv1.3 potassium channels in human T lymphocytes

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Distribution and lateral organization of Kv1.3 potassium channels – the dominant voltage-gated potassium channels – was studied in the plasma membrane of Jurkat T lymphocytes using electron microscopy, confocal laser scanning microscopy (CLSM) and fluorescence resonance energy transfer (FRET). Electron microscopy showed that the distribution of FLAG epitope-tagged Kv1.3 channels (Kv1.3/FLAG) significantly differs from the stochastic distribution. CLSM images showed that Kv1.3/FLAG channels and CD3 molecules were accumulated in largely overlapping membrane areas, indicated by a high cross correlation coefficient (C = 0.64). The molecular proximity between Kv1.3/FLAG and CD3 proteins was confirmed also by a high FRET efficiency (E = 51 %).
FLAG epitope-tagged Kv1.3 channels showed an uneven distribution in CTLs not engaged with target cells whereas the channels were recruited in the immunological synapse formed between CTLs and specific target lymphocytes. The redistribution of the channels was specific: transferrin receptor showed an even distribution in the membrane. Localization of Kv1.3 channels in the immunological synapse might have a role in regulating ion channel activities by signaling molecules accumulated in the immunological synapse.

P-35

Ex vivo multiphoton microscopy using endogenous signals in cardiac and vascular tissue

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We report two novel applications of multiphoton microscopy for pharmacological studies of unlabeled cardiovascular tissue.
First, we showed that multiphoton microscopy of unstained cardiac myocytes can be used to determine the sarcomere length with sub-resolution accuracy, owing to the remarkable contrast of the second harmonic signal originating from myosin filaments. A measurement accuracy of 20 nm is achieved, taking the sample variability into account. We used this technique to measure sarcomere contracture in the presence of saxitoxin, and results were in agreement with mechanical measurements of atrial tissue contracture [Boulesteix et al, Opt; Lett. **29**, 2031 (2004)].
Second, we characterized multiphoton microscopy of fresh unlabeled arteries. We performed simultaneous detection of two-photon-excited fluorescence (2PEF) from elastin laminae and second-harmonic generation (SHG) from collagen fibers upon 860 nm excitation. We showed that combined 2PEF/SHG images provide a highly specific, micron scale description of the architecture of these two major components of the vessel wall. We used this methodology to study the effects of a pesticide on the artery wall structure, using rats as animal models, and evidenced structural alteration of the vessel morphology.

P-36

Measuring protrusion forces of locomoting cells

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Cell migration is very important for cellular processes like wound healing or metastasis. Although much is known from the biological point of view on the actin-myosin machinery involved in cell migration, the exact mechanism of force generation is still unclear. One possible mechanism of force generation is the polymerization ratchet model. Here, thermal fluctuations of actin filaments are necessary for polymerization of actin filaments. Since this process effectively converts chemical energy in mechanical energy a protrusive force is generated. We have designed a cantilever-based instrument to measure directly protrusion forces at the leading edge of migrating cells. An AFM-cantilever oriented perpendicular to the substrate is deflected by a migrating keratocyte (epithelial cell prepared from trout scales). The deflection could be measured by video microscopy or at better temporal and spatial resolution using a position sensitive detector. The distance between cantilever and substrate was approximately 50 nm to guarantee that the leading edge of the lamellipodium was investigated. We will show first experimental results and discuss them in the context of existing theories.

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– Functional Cell Imaging –

P-37

Ultra-sensitive confocal fluorescence microscopy of C-reactive protein interacting with Fc-receptors

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C-reactive protein (CRP) has emerged as a powerful cardiovascular risk marker with a suggested pathogenic role in cardiovascular disease. The molecular basis of the interaction of CRP with cells remains unclear, however. Previous reports suggesting the low affinity IgG-receptor FcγRIIa as the major receptor for CRP have been criticized because of the use of anti-CRP antibodies, which interact with IgG-receptors via their Fc portion.
We have employed ultra-sensitive confocal fluorescence microscopy with gentle subunit labeling of the pentameric CRP for a quantitative study of the interaction of CRP with human Fcγ-receptors on cell surfaces. We find that CRP indeed binds to both FcγRIIa and FcγRI with low affinities in the micromolar range and slow association and dissociation rates. The slow dissociation over hours suggests multivalent binding of CRP to Fcγ-receptors and thus receptor clustering as a requirement for CRP binding.

P-38

Large-scale changes of intracellular calcium in response to GnRH and trh in mouse pituitary slices

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Pituitary hormone secretion depends on increases of intracellular calcium concentration $[Ca^{2+}]i$, either spontaneous or regulated by hypothalamic factors. Generally this has been studied in primary culture, where most forms of intercellular communication are disrupted. To visualize responses to secretagogues under conditions were anatomical relationships are more preserved, we recorded large-scale (half anterior lobe) $[Ca^{2+}]i$ fluctuations in adult female pituitary slices *in vitro*. Ca^{2+} imaging was performed after loading the slices with Fluo-4AM for 45 min. Slices were imaged under epifluorescence with a 10 x WI objective and a cooled CCD digital camera. Time-lapse sequences of up to 18 min were acquired. Bath application of TRH (100 nM) induces transient rises in $[Ca^{2+}]i$ in scattered cells throughout the slice, whose amplitude varies according to their position within the gland. This suggests differential sensitivity to TRH within the lactotroph/thyrotroph population. In contrast, application of GnRH (100 nM) generates, within a few minutes a $[Ca^{2+}]i$ rise in a small group of cells. A delayed rise in $[Ca^{2+}]i$, was seen in a different group of cells nearby, consistent with cell recruitment. This delayed response could be the result of differences in GnRH sensitivity or paracrine/electrical cell-to-cell communication. DGAPA IN-226403; 206004 and CONACYT 42662-Q.

P-39

Calcium signaling in newly formed growth hormone-secreting cells

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The increase in the intracellular calcium concentration is essential to the exocytotic process of secretion and many other functions (cell proliferation, differentiation...) in pituitary cells. In the pituitary, there is a continuing renewal of endocrine cells. How new endocrine cells maturate and integrate into pre-existing pools of fully-secreting cells remains unknown. The recently developed GH-GFP transgenic mouse allowed us to study GH cells in the intact pituitary tissue. We studied calcium signalling during maturation of GH cells in neonate transgenic GH-GFP mice. We used acute pituitary slices (230 μm thickness) from 1 to 4-day-old GH-eGFP mice, loaded with fura-2/AM. As the GFP chimera includes the GH signal peptide, we were able to observe that both mature (GFP mostly located in GH granules) and immature (GFP in the Golgi apparatus) GH cells displayed spontaneous calcium rises. This activity was blocked either in the absence of external calcium ions or by 5 mM EGTA, suggesting calcium activity was due spontaneous action potential firing. Strikingly, immature GH cells were characterized by shorter calcium spikes firing at higher frequencies. GHRH, a specific GH cell agonist, enhanced the frequencies of calcium spikes in both mature and immature GH cells. Large-scale synchrony of calcium spikes was detected in both mature and immature GH cells. Our data report the first characterization of calcium signalling in nascent GH cells.

P-40

Measurement of inter- and intracellular dye diffusion in the lens by two-photon flash photolysis

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Two-photon excited flash photolysis (TPEFP) is unique in its ability to release compounds with diffraction-limited resolution in three dimensions. We have used TPEFP to measure gap junction mediated cell-to-cell coupling of fiber cells in the mammalian lens. Rat lenses were loaded with CMNB-caged fluorescein and positioned on the stage of a confocal microscope. By focusing the light from a Ti::Sapphire laser into a selected fiber cell a point source of fluorescein was created. The movement of released fluorescein within and between fiber cells was monitored using an Argon ion laser. While diffusion of uncaged fluorescein was about an order of magnitude slower inside fiber cells than in aequeous solution, slower diffusion between cells could also be detected and could be explained by the gap junctions joining the cells behaving as a barrier to diffusion. By using a computer model, parameter fits to experimental data gave estimates for both intracellular and intercellular diffusion coefficients. From this analysis, the gap junctions in eye lens fiber cells permit exchange of low molecular weight compounds between cells at about 0.4% of the rate of free diffusion.
Supported by the Marsden Fund.

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– Functional Cell Imaging –

P-41

Differential polarization laser scanning microscopy on highly organized molecular macroassemblies

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With the use of a differential polarization laser scanning microscope (DP-LSM), constructed in our laboratory, we can obtain 3D information on the anisotropic organization of various biological samples. By measuring LD or FDLD (linear dichroism or fluorescence detected LD), and r, the anisotropy of the fluorescence emission, we can measure the preferential orientation of the absorption and emission dipoles, respectively. Linear birefringence (LB) also reveals the presence of anisotropically organized architectures. The degree of polarization of fluorescence emission (p) carries information on the spatial distribution of e.g. microviscosity of membranes. We demonstrate the usefulness of this novel imaging technique. Examples include the layer-by-layer variations of LD in plant cell walls, imaged by FDLD (Steinbach et al. in preparation). In wild type and mutant Drosophila embryos, r-imaging revealed variations in the local order of F-actin filaments in the O-ring channels, which depended on the presence of some key proteins (Gorjánácz et al. submitted to J. Cell Sci.). Similar r-images were recorded on various cytoskeletal structures. LB-imaging of granal chloroplasts explained their behavior in polarized beam-traps (Garab et al. Eur. Biophys. J. 2005). We also mapped the variations of p in lymphocyte cell membranes (Matkó et al. in preparation).

P-42

Combined FRET and anisotropy measurements synchronized with patch clamping

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Fluorescence resonance energy transfer (FRET) is often regarded as a "spectroscopic ruler" to determine the distance of molecules in the range of nanometers. It is used for binding studies and conformational studies of proteins. Distance determination by FRET requires knowledge about the orientation of the fluorophores in addition to the amount of FRET occurring. Recently, it was suggested to use linearly polarized excitation and to acquire information about the polarization of the acceptor emission to get better information about the real amount of FRET occurring. However, in some cases there might be pitfalls with this method due to changes in the rotational mobility as well as high fluorophore concentration and bleaching both of which diminish apparent anisotropy.

To overcome these problems, we have developed a method to acquire additional information about orientation. With every excitation we toggle between donor and acceptor wavelength and acquire simultaneously donor and acceptor emission, both in parallel and perpendicular polarization direction. This setup allows us to measure the fluorescence anisotropy of the donor and acceptor when directly excited and the loss of acceptor anisotropy due to FRET occurring which was performed on a membrane anchor domain and a G-protein coupled receptor.

Fluorescence measurements are synchronized with a patch clamp setup to gain information about conformation changes occurring on the voltage-gated, L-type calcium channel when switched between active and inactive states.

P-43

In vivo manipulation of morphogenetic movements in Drosophila embryos using nonlinear optics

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Embryo development involves complex cell movements that are tighly regulated by developmental genes. We recently reported that these movements may in turn mechanically induce the expression of developmental genes during *Drosophila* development[1]. Investigating these processes is technically challenging and requires novel experimental approaches. In this context, we show that the combination of femtosecond laser pulse-induced ablation and nonlinear microcopies appears as a powerful tool for modulating and quantifying morphogenetic movements in *Drosophila* embryos[2]. First, ultrashort pulse-induced ablation makes it possible to perform confined micro-dissections within developing embryos, resulting in a non-genetic modulation of distant morphogenetic movements. Then, the same laser source can be used to analyze native and disrupted morphogenetic movements both in GFP-labeled embryos using two-photon microscopy, and in unlabeled embryos using third-harmonic generation microscopy[3,4]. This methodology brings insight into the control of morphogenesis by revealing the correlation between tissue deformations and gene expression.

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[3] Débarre *et al*, Opt. Lett. (2004); [4] Débarre *et al*, Opt. Lett. (in press)

P-44

Auto-reverse nuclear migration in bipolar mammalian cells on micropatterned surfaces

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A novel assay based on micropatterning and time-lapse microscopy has been developed for the study of nuclear migration dynamics in cultured mammalian cells. When cultured on 10-20-μm wide adhesive stripes, the motility of C6 glioma and primary mouse fibroblast cells is diminished. Nevertheless, nuclei perform an unexpected auto-reverse motion: when a migrating nucleus approaches the leading edge, it decelerates, changes the direction of motion, and accelerates to move toward the other end of the elongated cell. During this process, cells show signs of polarization closely following the direction of nuclear movement. On the basis of our results, we argue that auto-reverse nuclear migration is due to forces determined by the interplay of microtubule dynamics and the changing position of the microtubule organizing center as the nucleus reaches the leading edge. Our assay recapitulates specific features of nuclear migration, while it allows the systematic study of a large number of individual cells. In particular, our experiments yielded the first direct evidence of reversive nuclear motion in mammalian cells, induced by attachment constraints.

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– Functional Cell Imaging –

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Single molecule imaging of nuclear transport in living cells and quantification of interactions

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We were able to clearly visualize single molecules inside cells and in living cells, using novel microscopy, Highly Inclined and Laminated Optical sheet (HILO) microscopy. GFP-tagged importin β, a carrier protein, and GFP-tagged cargo protein was examined during transport on the nuclear envelope. Image analysis of single nuclear pores showed that two point resolution of 70 nm was achieved. Kinetic parameters of the interactions between translocating molecules and nuclear pore complexes (NPCs) were obtained through quantitative analysis. Two types of binding site were found, weaker binding site, which gathers up to ∼100 molecules/NPC and concentrates molecules locally, and stronger binding site in the absence of RanGTP, with an affinity that changes drastically upon translocation ability. Accessibility of importin β to the stronger binding site is critical for NPC translocation. In the presence of RanGTP, the stronger binding site disappears and the active site appears. Translation rates into the nucleus were obtained and corresponded well with the retention time of single molecues. The correlation coefficient shows that the maximal binding was 8 (or possibly 16) molecules/NPC. Based on these findings, we propose a novel model of NPC translocation. Thus, single molecule imaging a powerful technique to quantify dynamics and kinetics of molecular interactions and to elucidate molecular mechanisms inside cells.

P-46

Study of cell line by Atomic Force Microscopy

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New trends in application of Scanning Probe Microscopy give us ability to scan living cells directly in their ingenuous surroundings. The aim of presented study is surface picture of cell sample in liquid surroundings of nutrient media. The minimal forces between tip and surface of the sample, avoid damage of biological preparation. One of this application is Atomic Force Microscopy, which give us possibility to picture living cells with their non-damaged biological activities. Non-contact or tapping mode use in biological application of AFM. As biological materials we used cell line G361 a T98. Own works solves problems and artefacts presented during scanning of biologic materials located in liquid. Scanning of this materials is limited by construction AFM. Is the aparatus replenished with inverse optical microskope, now it use observe work of tip in individual scanning of cell. Tip movement tears away individual cells from cover of substrate. The results in disconection between tip and cell. For preparate fixation is necessary to deal aid physical-chemical variants. It was developed method for scanning of sample in liquid surrounding, which removes this deficits. This Work was supported by the Ministry of Education of Czech Republic MSM 6198959216.

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Fluorescence cross-correlation microscopy shows cytoskeleton-independent co-mobility of MHC I and II

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MHC class I and II glycoproteins are responsible for presenting antigens to T cells. We and others have shown previously that oligomerization of MHC I molecules increases the efficiency of antigen presentation. In addition to homoassociation of both MHC I and II molecules, their heteroassocitation has also been demonstrated in B and T lymphocytes by fluorescence resonance energy transfer. In this study we used fluorescence correlation and cross-correlation microscopy to investigate the mobility and co-mobility of MHC I and II molecules in JY B lymphoma cells. In intact plasma membranes the diffusion coefficient of MHC I molecules was an order of magnitude lower than in blebs. Cross-correlation measurements on MHC I and II molecules labeled by Alexa 488- and Cy5-tagged mAbs resulted in positive cross-correlation amplitudes implying their co-mobility in intact membranes and in blebs. Our results suggest that interaction and aggregation of MHC I and II does not depend on the cytoskeleton.

P-48

Role of GM1-positive membrane microdomains in signaling by the PDGF receptor in glioblastoma cells

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PDGF receptors play an important role in the proliferation of glial tumors. Key events in their activation are di- (oligo)merization followed by transphosphorylation and downstream signaling. Our aim was to reveal how cell confluence – dependent PDGFR activation is regulated by the molecular environment of receptors in the cell membrane using immunofluorescence, video- and confocal microscopy and digital image processing The glioblastoma lines A172 and T98G expressed mostly PDGFR beta. The number of receptors in the cell membrane increased as cell cultures reached confluence. Parallel to this, calcium responses evoked by PDGF were 2-phased and prolonged in an increasing portion of cells. Receptors showed a non-random, clustered distribution in the cell membrane. The overlap of receptor clusters with CTX-B-labeled lipid rafts was substantial. The cross-correlation coefficient characterizing the overlap increased with cell confluence. Furthermore, receptors showed higher relative phosphorylation in rafts than outside rafts. Crosslinking of the lipid rafts by CTX-B at 37 C led to the aggregation of lipid rafts and sequestration of PDGFR clusters from them. Reducing the cholesterol content of the cell membrane by methylbeta-cyclodextrin dispersed lipid rafts and PDGFR clusters, decreased their overlap, and almost completely abolished phosphorylation- and calcium-response to PDGF. We conclude that raft localization of PDGFR has a functional consequence and is linked to regulation of proliferation as cells reach confluence.

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– Functional Cell Imaging –

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Accurate estimate of biomolecular concentration in living cells at high spatial resolution

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The concentration of native biomolecules (e.g., proteins, cofactors) can be used as a reporter for health and disease diagnoses. Two-photon (2P) fluorescence imaging is a noninvasive approach for qualitative imaging of these biomolecules. However, quantitative estimate of the concentration of these biomolecules requires in-depth understanding of how the heterogeneous cellular environment might affects their fluorescence properties. Here we present a 2P-fluorescence-based method for accurate analysis of molecular concentration imaging using a combination of steady-state and time-resolved 2P-fluorescence lifetime microscopy. As proof of this concept, we use human breast cancer cells, stained with a mitochondrial marker Rhodamine 123, as a model system. The advantage of our approach relies on the sensitivity of fluorescence lifetime to the cellular environment and molecular structure. Monitoring the variation in biomolecular concentration, using image correlation analysis, can be a useful tool in medical diagnosis and for monitoring physiological changes in living cells.

P-50

Comparison of three-dimensional tracking of single granules in live PC-12 cells

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Abstract-Total internal reflection fluorescence microscopy (TIRFM) permits the selective illumination of a $\sim$200 nm thin optical section at the bottom of the cell adhering to the coverslip, so can only be employed to study granules right underneath the plasma membrane. In order to surmount the inherent limitation of TIRFM and obtain the movement of granules deeper inside the cell, we constructed an imaging system of deconvolution wide-field fluorescence microscopy (WFFM). Both techniques were applied to follow the three-dimensional mobility of single secretory granules in live neuroendocrine PC-12 cells, and a comparative study was carried out on evaluating the performance of them. The comparisons revealed that the results of WFFM were analogous to those of TIRFM, most acridine orange-labeled granules were found to travel in random and caged diffusion, and only a small fraction of granules traveled in directed diffusion. Furthermore, the size and 3-D diffusion coefficient of granules, obtained by these two techniques, yielded the same value. Together, our results demonstrate the potential of the combination TIRFM and WFFM in tracking long-termed motion of granules throughout whole cells.

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– Modelling Complex Systems –

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Molecular simulations of the stability of an alanine octapeptide confined in AOT reverse micelles

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We have used Molecular Dynamics (MD) simulations in NPT ensemble (T=300K and P=0.1MPa) for small sized AOT Reverse Micelles (RM) (W_o=[H_2O]/[AOT]=5 and 7). We have used the ORAC MD package with/without a zwitterionic alpha-helix alanine octapeptide confined in the water core. We examined the time evolution of its conformational change in these systems and in bulk water. We have computed the root-mean-square deviation from the initial structure and stability of intra H-bonds in alpha-helix structures and analyzed the RM structures in presence/absence of the peptide: we have calculated the size of the water core and of micelles, the radial mass density profiles, the headgroups hydration, the micellar water diffusion etc. The results indicate that the aggregate structural properties were significantly modified for the small RM, in contrast to the larger ones. When the RMis small the alpha-helical secondary structure of the peptide is conserved and stable. As the quantity of water increases, we observe an unfolding process. To test if the primary hydration of the polar headgroups of the surfactant can favor the stability of the peptide in the small RM,we removed the AOT headgroups. This results in the loss of the peptide structure. These MD are in qualitative agreement with experimental observations which indicate that hydration is a crucial parameter for the stability of peptides/proteins confined in small-size RM.

P-52

The effect of self-organizing at binding of ligands with macromolecules

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The adsorption process is represented as transition of absorbable particle in a free potential hole of adsorption center from a potential hole disposed close to the adsorption center. We consider that in the process of the adsorption the ligand deforms the potential profile of adsorption center in such manner, that the potential hole becomes somewhat deeper. At reversible adsorption after a desorption the energy profile of adsorption center will relax to the initial state. If the relaxation time is longer, than the mean time between two subsequent arrivals of ligands in the adsorption center, the structure changes of the adsorption center will promote more effective binding of the ligand with the adsorption center. This circumstance leads to self-organizing effect of the adsorption of ligands. The present work is devoted to the theoretical investigation of the adsorption of ligands on macromolecules when the adsorption of ligands and the changing of the structure of the adsorption center are described in a selfconsistent way. Such an approach allows to obtain a number of properties of the binding center: plurality of stationary states at binding, realization of trigger and hysteresis behavior of binding modes. It is also shown that considering of adsorbtion centre deformation leads to a S-shaped adsorbtion curve.

P-53

The swelling of Tomato Bushy Stunt Virus: a multi-approach study

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Time-resolved Small-Angle X-ray and Neutron Scattering (SAXS and SANS) in solution were used to study the swelling reaction of TBSV upon chelation of its constituent calcium. SAXS intensities comprise contribution from the protein capsid and the RNA moiety while neutron scattering is essentially due to the protein capsid. Cryo-electron micrographs of compact and swollen virus were used to produce 3D reconstructions of the initial and final conformations of the virus. While compact particles appear to be very homogeneous in size, solutions of swollen particles exhibit some size heterogeneity. A procedure has been developed to compute the SAXS pattern from the 3D reconstruction for comparison with experimental data. Cryo-electron microscopy thereby provides an invaluable starting (and ending) point for the analysis of the time-resolved swelling process using the scattering data.

P-54

On “exotic” interactions in biomolecular systems

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Most of our reasoning regarding the interactions that maintain the structure of biomolecular systems is associated with the formation and disruption of classical hydrogen bonds. This view has proven to be quite convenient since such hydrogen bonds can be directly inferred from crystal and NMR structures. However, a certain amount of evidence coming from experimental and theoretical studies indicates that, not only these classical hydrogen bonds participate to the transformation processes of biomolecular systems but also other interatomic forces are involved. Among those, one can cite CH…O/N hydrogen bonds, hydration forces, cation-π interactions and halogen bonds. This list is not restrictive. If we want to improve our understanding of the molecular recognition (including folding) processes associated with biomolecular systems, all “exotic” interactions have to be taken into account. This is also crucial for the design of reliable dynamical models obtained through molecular dynamics simulation techniques. This will of course increase the complexity of the models but also enhance their pertinence. A few examples related to molecular dynamics simulations of RNA systems will be discussed.

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– Modelling Complex Systems –

P-55

A new implicit solvent model for proteins

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Electrostatic studies of proteins are often performed using Poisson – Boltzmann (PB) solvers, which use a continuum model of the solvent, as implemented by Delphi, UHBD or APBS for example. PB Equation assumes arbitrary dielectric constant values for the protein interior (between 1 and 8), while a value of 80 is assigned to the outside. By construction, PB does not give information about solvent density around proteins. In order to circumvent this problem, we present here a Generalized Poisson–Boltzmann Equation (GPBE) obtained by minimizing the free energy functional of the system. It automatically adjusts the solvent density (represented as dipoles of finite size) as well as mobile ions density in response to the electric field of the solute. The GPBE, solved by a nonlinear Multigrid algorithm, gives the ion and dipole density at every point of a grid and also the dielectric profile of the system. Obtaining this dielectric profile, as well as the solvent density, are the main originalities of this model. The calculation of the water density allows us to localize water preferred-sites around protein structures. Examples of application for membrane and homodimeric proteins will be given.

P-56

Biophoton emission from lichens: an indicator of the physical basis of life

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A sample of lichen spontaneously emits photons of constant average flux for many hours. The photons are mainly in the visible range and are called biophotons. The photon flux fluctuates probabilistically and the probabilities of detecting various numbers of photons in a measuring bin have definite values. The probabilities determine four parameters of a quantum squeezed state. The parameters are new attributes of the sample. The attributes have nearly same values in measurements with different bin sizes and in various spectral decompositions of a biophoton signal. It is an evidence of the quantum nature of the signal. The evidence is found in signals of any lichen species. A biophoton signal probably emanates from a collage of scintillating quantum patches of nucleotides and it contains information of holistic functioning of a living system. The holistic response of living system to light stimulation is again a photon signal of unusual features; prominent among them are ultra weak strength, non-exponential decay, long non-decaying tail, sensitivity to many factors and quantum nature. It is also called biophoton signal. Its unusual features are described in a phenomenological model of four parameters. The parameters are other new attributes of a sample, which can detect changes in the sample caused by a psychic healer from a distance. The new attributes opens up new dimensions of exploration for understanding the incomprehensible features of living systems.

P-57

Modeling tumor growth in vitro: A Kinetic Monte Carlo study

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We study the spatio-temporal growth dynamics of cell structures. Modeling a two-dimensional monolayer of in vitro tumor cells, we use a Kinetic Monte Carlo method to analyse the experimentally accessible parameters of tumor growth. By employing a simulation based on a Voronoi grid, we avoid artefacts of regular lattices with symmetries, as well as problems of computationally much more expensive off-lattice models. Including cell-biophysical and cell-kinetic properties in our model, we show that our simulations satisfy the basic dynamic conditions observed in experiments. Concentrating on the macroscopic properties of tumor growth we outline how such a model tool may be used to predict changes in the growth kinetics and in the spatial structure of cell populations if selected model parameters are varied. In particular, the main characteristic properties like the growth velocity and the morphology of the cell population in terms of the roughness evolution are analysed.

P-58

Determination of Ca^{2+} currents underlying Ca^{2+} puffs in Xenopus oocytes using a heuristic model

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Recent advances in cell imaging techniques have revealed the existence of a wide range of events involving the release of Ca^{2+} from internal stores. A complete description of these signals requires a detailed knowledge of the magnitude and kinetics of the underlying Ca^{2+} flux. These can not readily be inferred from fluorescence images since the dye and the endogenous Ca^{2+} buffers and pumps that are in the cell affect the spatio-temporal dynamics of the released Ca^{2+} ions. We have recently introduced a method for obtaining the released current that requires few assumptions and is largely model-independent. In this work we modify it slightly and apply it to characterize puffs, which are the result of Ca^{2+} release through clusters of IP3Rs channels, in Xenopus oocytes. The current estimates that the method provides are limited by the time and space resolution of the experiments. For this reason, lower bounds of the current can be obtained. In particular, in the case of puffs we obtain current values that range between 0.4 and 1.2 pA which are consistent with either a relatively small number of simultaneously open channels or with a very small single channel current.

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– Modelling Complex Systems –

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Mathematical modelling of photosystem II functioning

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A mathematical model of the electron transfer reactions in the Photosystem II supra-molecular complex is designed. The model includes the electron carriers between the oxygen-evolving complex and the plastoquinone pool. Specialized computer software is developed that allows the automatic construction of the differential equations describing the transitions between the redox states of the electron carriers. The model is tested using the luminescent characteristics of Photosystem II – prompt and delayed chlorophyll *a* fluorescence transients from dark to light-adapted state. By fitting the model curves to experimental ones by the same software, the values of the rate constants of the particular electron transfer reactions are assessed. An approach to fitting is proposed that avoids overparameterization and allows the acquisition of correct values of the rate constants by the simultaneous fitting of several types of experimental curves (prompt and delayed chlorophyll *a* fluorescence) and curves obtained at different experimental conditions. The method allows the evaluation of the rate constants in native plants and in plants under the influence of different environmental factors in *in vivo* and *in situ* measurements. For example, as it is expected, the growing of barley plants at different light intensities causes the change of the parameter that describes the Photosystem II antenna size.

P-60

Uncovering the overlapping community structure of complex networks in nature and society

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Many complex systems in nature and society can be described in terms of networks capturing the intricate web of connections among the units (proteins, genes, people, etc.) they are made of. A question of great current interest is how to interpret the global organization of such networks as the coexistence of their structural sub-units (communities) associated with more densely interconnected groups of nodes. Identifying these communities is crucial to the understanding of the structural and functional properties of networks. The existing methods used for large networks find separated communities, however, in most real networks the communities are believed to overlap with each other at a great extent. Here we introduce a new approach to determine the interwoven sets of overlapping communities of large real networks [1]. Among other examples, we demonstrate the power of our method by identifying the communities of proteins in the protein-protein interaction network of yeast, which then, for example, allows us to make predictions for yet unknown functions of some proteins.

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P-61

Component analysis of the fluorescence spectra of simple and polymeric phenolic compounds

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Fluorescence spectra of simple (hydroquinone) and polymeric (lignin model polymer - DHP) phenol compounds were mathematically analyzed by band decomposition method. Emission spectra were obtained by stepwise varying excitation wavelength with 5 nm step, starting from the excitation maximum to 465 nm. Decomposition was performed by nonlinear fitting of all three Gaussian parameters: area, width and position. Position of all components in a series was treated as a random variable and its approximate probability distribution (APD) calculated from a series of histograms with increasing number of abscissa intervals. Emission spectra of hydroquinone were measured in methanol. DHP was studied in methanol/chloroform solution and solid suspension in water, in order to compare influence of different state solution/solid. The aim of this study was to see whether the component analysis might be used as a theoretical tool in structural characterization of complex (polymeric) phenolic compounds.

P-62

A model of RecA-mediated homologous recombination

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RecA protein is a key part of the homologous recombination machinery in *E. coli* bacteria, promoting strand exchange between DNA molecules bearing identical or very similar sequences (homologous molecules). A key step in the process is the homology search, where a RecA-single stranded DNA filament has only several minutes to find its homologous double stranded DNA counterpart amongst a sea of heterologous strands and then perfectly align and pair with it. We present a model of this process that accounts for both the polymeric nature of the DNA and filament, as well as the fact that initial recognition can take place anywhere along the sequence [1]. A key feature is the assumption that that longitudinal fluctuations in the base-pair spacing are responsible for overcoming the difference in rise [2]. Using perturbation analysis of the multiscale, first passage time problem, we derive an analytical expression for the average search time. Our formula agrees well with published experimental results [3], and our results strongly suggest that recognition requires a seed of 3 base pairs to align with its counterpart in order to stabilize the three-strand complex.

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– Modelling Complex Systems –

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Identification of synergetic property of biological dynamic system (BDS)

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Compartmental-clusters theory provides the modelling of BDS. The identification of model's parameters is based on special theories of identification of BDS. According to these theories we can identity the synergetic property of different BDS. The work presents procedure that provides the identification of synergetic property of respiratory neuron network (RNN) as an example of BDS.
According to compartmental approaches every BDS has compartmental stricture. Between every compartment the connectedness may be presented by coefficient a_{ij}. The coefficient includes on matrix A. So the matrix A and its eigenvalues present the biological property of neuron network and its specifically functional condition. The basic model of irreducible (with cyclic structure) neuron network with compartmental property has a form:

$$\begin{matrix} dx/dt = AP(y)x - bx + ud \\ y = c^T x \end{matrix}$$

, where $x, d \in R^m$, $A = \{a_{ij}\}_{i,j=1}^{m}$, $a_{ij} > 0$ if $i \neq j$, $P = diag\{p_j(y)\}_{j=1}^{m}$, $y \in R^1$. The stage-vector x presents the activity of RNN on different conditions.The final procedure is connected with calculation of synergetic degree of RNN by formula: $\chi = k\left(\sum a_{ij}^{*}(<0)\right) \times \left(\max a_{ij}(<0)\right)$
If $\chi \to 0$ the synergetic interaction in RNN increase. Other way for $\chi >> 0$ the synergetic property in RNN is loosed. Such procedure was used for RNN and the value of synergetic degree was calculated. The discussion of biological result was presented.

P-64

Theory of fazaton brain and method of identification its models

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The identification of normal and pathological state of human organism can be based on new modern system criteria. Now it is evident that neuromotor's complex, neurotransmitter's and vegetative neuron system have common roots. It is possible to identify the existence of common root of regulation of these three systems by brain structure named by us fazaton brain (FB). The cooperation of neuromotor complex, neurotransmittor complex and vegetative neuron system are based on regulation of such systems by fazaton brain.
Now it is evident that tonic motor system has common root with parasympathetic vegetation nervous system. Vice versa the phasic motor system has common root with sympathetic vegetative nervous system (with catecholaminergic neurotransmittor mechanism). The method of FB identification and computer-aided modeling were discussed.
Our software and hardware provides the registration of FB stage on phase space for pathological and normal state of human organism. Such identification with computer using we introduce to different clinic (surgical, therapy, endocrinology, neuropathology) of Surgut. Now we use specific program for diagnostic of FB in medicine.

P-65

Viral ion channels: small proteins with a big effect

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Ion channels are membrane proteins which assemble within the lipid bilayer generating a water-filled pore through which ions can flow. Viral genomes encode ion channels with a length of around hundred amino acids. These channels adopt a range of topologies and are increasingly viewed as potential drug targets and for application in Bio-nanotechnology. A summary of viral ion channels will be given.
Vpu from HIV-1 (81 amino acid) is used as a test case. It has a short transmembrane (TM) domain, responsible for channel activity, and a larger cytoplasmic domain, involved in CD4 receptor degradation. MD simulations are used to address the mechanism of function of the TM domain of this protein. Forcing ions through the pore delivers a cinematic picture of the movement and the dynamics of the water molecules within the pore. First results of the modelling of full length Vpu will be shown. All simulations with full length Vpu indicate that the cytoplasmic part of the protein orients along the membrane surface.

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Analysis of the mechanism of activating peripheral nerves by a transverse electric field

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The classical cable function has been used to represent the response of peripheral nerves stimulated by an external parallel electric field, which predicts that excitation occurs near the maximum of the negative first spatial gradient of electric field in the direction of the never. But this function can not describe the excitation of peripheral nerves stimulated by a perpendicular electric field induced by pulse magnetic field. In this paper, responses of the Ranvier nodes to a transverse-field are further investigated by mathematic simulation and in vitro experiments. This simulation demonstrates that, under perpendicular electric field stimulation, the responses of Ranvier nodes evoke a two-stage process including an initial polarization and the actual change of the transmembrane potential. This simulation demonstrates that the peripheral nerve excitation results from the net inward current along a radial direction of the Ranvier node driven by an external field. Based on the two-stage process, a novel model is introduced to describe peripheral nerves stimulated by a transverse-field, and the classical cable function is modified. The new model and the improved cable function are verified by several in vitro experiments. They can be used to represent the response of peripheral nerves stimulated by an arbitrary electric field.

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– Modelling Complex Systems –

P-67

Active transport in disoredered microtubule networks: the generalized random velocity model

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The motion of small cargo particles that are carried by microtubule associated motor proteins in disordered microtubule networks is investigated. Different network topologies in two and three dimensions are considered, one of which has been recently studied experimentally *in vitro* by M. Elbaum and coworkers. A generalization of the random velocity model is used to calculate the mean square displacement of the cargo particle. We find that all cases fall into the class of enhanced diffusion that is sensitive to both the dimensionality and the topology of the network. Yet, in three dimensions the motion is very close to simple diffusion. When the thermal diffusion in the bulk solution is included, no change in the asymptotic time behavior is found, as expected, but the prefactors are sensitive to the physical parameters of the system.

P-68

A simple model symbiosis using nutrient-deficient Escherichia coli

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In natural habitats, many organisms have symbiotic networks and the interactions among them have been investigated. However, how the interaction affect the stability against environmental change or others remains unclear as it is unrealistic to add systematic perturbations to the network and carry out a long term observation under a fixed boundary condition. In addition, the molecular genetic knowledge behind the network is too complex. To get insights on the stability and evolvability of the network, the first step would be to construct a simple experimental model. We made a simple system composed of two *Escherichia coli* strains with different nutrient requirements. By deleting a gene from the *E. coli* genome, we obtained four nutrient-deficient strains. The 67% of all pairs of the four strains grew on minimum agar medium as leaking the nutrient the others requires. To see the stability of this system, two strains of them were labeled by GFP and RFP, respectively, co-cultured in liquid minimum medium and analyzed the existing ratio of them by flow cytometry. They co-grew only when their growth rates were suppressed to some extent. It suggested that interaction to establish mutualistic relationship came out when their growth rate was down causing the lack of nutrients. In fact, when one of the strains grew at the ordinary rate, the co-growth has not been observed. These results suggest that the physiological change under the suppressing environment is crucial to the stability of the symbiotic network.

P-69

Characterizing electron transfer in photosystem II

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We explored electron transfer (ET) processes in photosystem II (PSII) computationally based on the crystal structures (1,2) solving the Poisson-Boltzmann equation. The accuracy of our approach was recently demonstrated calculating heme RP of cytochrome *c* in bacterial RC (3). We considered the ET processes involving chlorophylls (Chl) and quinones as well as the redox-active tyrosine$_Z$ (Y_Z) and Chl P680 dimer (4,5). The energetics of these ET compare favorably with known experiments on cofactor RP and ET rates demonstrating how proteins tune cofactor RP. The computations explain why the quinone RP in PSI are so high while they are so low in PSII and bRC. They also provide large values of the chlorophyll P680 RP in PSII, which are needed for oxidation of Y_Z necessary for water oxidation at the Mn-cluster. The energetics of the ET from Y_Z to the P680 dimer can be understood by proton shuffling between Y_Z and D1-His190 known as so-called proton rocking mode.

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P-70

Capsid surface properties analysis of RNA F-specific bacteriophages

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RNA F-specific bacteriophages specifically infect bacteria having sexual pili (F-pili). They consist of a positive single stranded RNA and a non-enveloped capsid composed of only one protein coded by one gene. This family of bacteriophage (*leviviridæ*) is often used as a model of behavior study of pathogenic enteric viruses in a hydrous medium. Presently, very few data relating to properties of capsid surface of these bacteriophages are known. Combining cryo-electron microscopy and molecular modeling from stocks of purified viruses, it is now possible to have precise information on positive and negative amino acids repartition and hydrophobic residues topology. These results will thus allow to specify conditions (pH, ionic force...) supporting adhesion and aggregation phenomena of viral particles in solution.

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P-71

A discrete model of the regulatory network defining the Wingless boundary in the Drosophila wing

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The dorsal-ventral margin of the Drosophila wing arises from a boundary of cells expressing wingless during the early larval stages (for a review see Irvine & Vogt 97). We have modelled the intercellular gene network defining the Wingless boundary using the logical generalised formalism (Thomas & d'Ari 90, Chaouiya et al. 03). The model involves seven genes in four interacting cells, where two of the genes are multi-value. The resulting dynamics from the simulation is represented in a graph, where vertices represent genetic states and edges represent genetic transitions. In this poster, we define formally, the steady states, the basins and the bifurcations in the dynamical graph. Then, we show the results regarding our model of the network defining the Wingless boundary. Finally we discuss the biological implications of our results.

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P-72

Quantitative prediction for two-dimensional bacterial genomic displays

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Two-dimensional bacterial genomic display (2DBGD) is a simple technique that allows one to directly compare complete genomes of closely related bacteria. It consists of two phases. First, polyacrylamide gel electrophoresis (PAGE) is used to separate the DNA fragments resulting from the restriction of the genome by appropriate enzymes according to their size. Then, temperature gradient gel electrophoresis (TGGE) is used in the second dimension to separate the fragments according to their sequence composition. After these two steps, the whole bacterial genome is displayed as clouds of spots on a two-dimensional surface. 2DBGD has been successfully used to distinguish between strains of bacterial species. Unfortunately, this empirical technique remains highly qualitative. We have developed a model to predict the location of DNA spots, as a function of the DNA sequence, the PAGE and TGGE conditions, and the nature of the restriction enzymes used. This model can be used to optimize the procedure for the type of bacteria being analyzed.

P-73

Electrical parameters of fruit and vegetable slices of different sizes

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The electrical impedance spectrum of apple, potato and carrot slices of different size (from 30 mm x 3 mm x 3 mm till 30 mm x 60 mm x 60 mm) were obtained with a HP precision RLC meter in a frequency range of 30 Hz up to 1 MHz at 1 V voltage. The spectra were measured between two copper covered gold pin-electrodes punctured into the slices in distance of 20 mm, 10 mm, 5 mm and 2 mm, respectively. The electrode polarization was eliminated. The impedance spectrum of distilled water also was measured in the same geometrical arrangement as the slice geometry. Comparing the spectrum of slices to the spectrum of distilled water the dielectric constant and the dielectric loss of the tissues were evaluated. The dielectric constant of apple, carrot and potato was decreased from values of 75, 78 and 70 to values of 70, 75 and 65, respectively, while frequency increased from 30 Hz to 1 MHz.

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P-75

Stable stochastic dynamics in yeast cell cycle

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In cell cycle a cell should bear intense noise caused by the rapid change of the number of molecules in the range of 0 to $\sim 10^3$. The stochastic dynamics of budding yeast cell cycle is modeled by a master equation to examine whether the stable cyclic behaviors should persist in the noisy dynamics.

In our model of the cell-cycle network, each of fourteen kinds of proteins is classified by the state of chemical modification: phosphorylation/dephosphorylation and ubiquitination. The network dynamics is described by a probability distribution P as a function of the activator-promoter binding state at each gene and the number of mRNAs and the classified proteins. Assuming that reactions in the network can be approximated as Markovian processes, and that P is factorized into the product of Gaussian functions, the master equation for P is reduced to a set of approximately 300 differential equations for the average and the dispersion of the number of biomolecules.

Temporal changes in distributions of the number of molecules are numerically followed by solving differential equations. Starting from broadly distributed initial conditions, trajectories rapidly converge in the range observed in experiments and then display the limit-cycle like behavior. The fluctuation in the number of proteins is strongly suppressed to make the distribution Poissonian with the relaxation time depending on the degradation rate of ubiquitinated proteins, suggesting that the ubiquitine-mediated rapid proteolysis contributes to the stability of the system.

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P-76

Stochastic fluctuation and relaxation in a genetic feedback loop

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Large stochastic fluctuation due to the small number nature of biomolecules in a cell is essential in gene expression. Many attempts have been made to estimate the variance of the number of synthesized proteins, $\langle(n(t)-\langle n(t)\rangle)^2\rangle$, where $\langle\ldots\rangle$ implies the average over the cell population. Sufficient information on the temporal change of individual cells, however, can not be obtained from those analyses based on the single time statistical quantities. Deeper insights into the time evolution of cells should be gained by developing a new method to calculate the two-time correlation $\langle n(t)n(t')\rangle$ and the response function $d\langle n(t)\rangle/dX(t')$, which accompanies the change of the reaction condition X. We develop a theory to describe the time evolution of individual cells by means of the path integral formulation and the numerical stochastic simulation using the Gillespie algorithm. We also discuss the fluctuation-dissipation relation between the time correlation and response in cell.
We take as an example one gene negative feedback loop, in which the gene product itself is a repressive regulatory protein for its gene. The master equation describing the stochastic process in this system can be converted into the equivalent form of quantum mechanics (Sasai and Wolynes, PNAS,**100**, 2374-2379, (2003)). This mathematical analogy is convenient to develop the path integral formulation, in which classical path corresponds to the deterministic reaction equation and the semi-classical fluctuation around the classical path is taken into account.

P-77

Global structural and mechanical properties of biomimetic membranes

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Over the last decade or so global x-ray and neutron diffraction data analysis of biological model membranes has received considerable amount of scientific attention. The reason is that this technique allows the determination of global structural as well as elastic membrane properties close to conditions found in biological membranes. Henceforth, local changes to the bilayer by e.g. interactions with a membrane active compound can be related to a global adjustment of structure or elasticity. In turn, global membrane properties affect their local susceptibility for such an interaction. We will review the presently developed techniques both for diffraction from vesicles and solid supported multibilayers and discuss advantages, disadvantages and challenges. We will further present a series of application examples both for single-component and multi-component membranes.

P-78

Neural network analys of results medico-psychological inspection of the miners

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Object: 100 miners having the underground experience of work in extreme conditions.
Methods: Psychodiagnostic techniques of revealing adaptation and person of properties, neural network analys of the data - method of construction of self-organizing cards by Kohonen.
Results: 23 men the expressed emotional intensity is marked. Separate attributes PTSD are fixed in 4 men. 6 men is diagnosed astenia. 6 men mark at themselves a moderate psychological pressure. The high level of an alarm is revealed in 2 men. 10 men the easy depression and 7 men - subdepression a conditions is fixed. The entrance layer of a network contained 9 neurons with radial function of activation, the target layer consist of three neurons, in which the classification of analyzed objects (in a target layer was carried out was tested from 2 up to 25 neurons, thus most information there was a classification in 3 clusters). In result clusterises was received model which has divided analys set on clusters V1 (53 men), V2 (24 men), V3 (23 men). Among the examinees two groups of the people are allocated: I - group, which condition practically on all parameters (except for a scale of emotional intensity) is worse - cluster V3; II - group, which condition practically on all parameters is more safe - clusters V1, V2. Surveyed, referred to clusters V1 and V2 differ only on a scale of emotional intensity - in cluster V2 it above.
Conclusions: The high effectiveness of neural networks for cluster analysis of outcomes of research is shown.

P-79

Theory of bio-energy transport in living systems and its properties

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Many biological processes, such as, muscle contraction, DNA reduplication**,** neuroelectric pulse transfer on the neurolemma and work of calcium pump and sodium pump, etc., need supply energy, which comes mainly from the energy released in hydrolysis of adenisine triphosphate(ATP) binding usually to a specific site on the protein. Thus there are always bio-energy transport along protein molecules in the living systems. We proposed a new soliton model with quasi-coherent two-quantum state and containing various interactions for this transport on the basis of molecular structures of proteins. We find out the properties of this bio-energy transport by this theory. The soliton is formed by self-trapping state of amide-I quantum (C=O stretching vibration), arising from the energy released by ATP hydrolysis, interacting with distortion of the amino acids, and can move over a macroscopic spacing, retaining its wave shape, energy, momentum with constant velocity. We get from this model that the soliton is thermally stable and very robust against the thermal perturbation and disorder effects of molecules structure and has enough long lifetimes (about 100-300Ps) in periodic and nonuniform proteins at biological temperature 300K by analytic way of nonlinear quantum perturbation and numerical simulation of fourth order Runge-Kutta ways, respectively. Therefore the soliton is a carrier of bio-energy transport in proteins, this new model is correct and appropriate to the protein molecules.

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P-80

Connectivity of the signaling network triggered by neurotrophic factors and ECM in sensory neurons

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Neurotrophic factors and laminins are important regulators of post-traumatic regeneration in the nervous system. Cyclin-dependent kinase 5 (Cdk5) regulates cytoskeleton mobility and mediates the effect of neurotrophic factors on axonal growth in various types of neurons. Here we show that NGF, GDNF and neurturin activate axonal growth in mature dorsal root ganglion neurons in the absence of Cdk5 activity as the effect of these neurotrophic factors was not affected by 50uM of roscovitine. On contrary laminin-dependent outgrowth in the absence of neurotrophic factors was fully blocked by roscovitine. GDNF- and laminin-dependent types of axonal growth also have different sensitivity to src inhibitor SU6656. We use Boolean networks formalism to analyze differential contribution of neurotrophic factors- and laminin-triggered pathways to the converging signaling network.
Our results demonstrate that there are two noncrosstalking pathways mediating the signalling from laminin and neurotrophic factors in sensory neurons. Response of the whole cell to the ligand stimulation can be described by a network of a few Boolean elements. The two signalling pathways act to discriminate between two combinations of ligand inputs: laminin alone versus laminin plus a neurotrophic factor. The two pathways act on different time scale. The two pathways are not additive if activated simultaneously. Using Boolean networks formalism we define explicit limitations on connectivity of the signaling network.

P-81

Dynamics of bent DNA studied through computer simulation

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In some nucleic acids permanent bents have been identified. In addition, it is known that nucleic acids show a certain degree of flexibility. Different experimental techniques, based in solution properties, for example transient electric birefringence, have been used to characterize the rotational dynamics of DNA and RNA in solution. But full interpretation of experimental results need of complex analytical theories which in some cases are not available. In those cases methodologies based on computer simulation appear as a valuable tool. We present a computer simulation based methodology that using Brownian dynamics and rigid body hydrodynamic modelling can help us in the interpretation of experimental data to characterize permanent bents and flexibility of nucleic acids. This general methodology is applied, using a hydrodynamic model, to a system example: the B-DNA with possible flexible bents, and the interpretation of experimental data obtained by electro-optical techniques like transient electric birefringence is exposed.

P-82

Collective Phenomena and Jamming in Intracellular Transport

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Intracellular transport driven by motor proteins is a complex process whose biophysical properties are far to be understood. Theoretical studies on simplified systems can reveal interesting collective phenomena that can be relevant for intracellular transport in physiological conditions.
On the basis of driven stochastic processes far from equilibrium, we propose a simple model for transport driven by motors moving on a single cytoskeletal filament in contact with the cytoplasm.

P-83

A Computational Study Of Factors Effecting The Spatial Spread Of Calcium Sparks In Cardiac Myocytes

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Local calcium release events at the t-tubules of heart muscle cells, called Ca^{2+} sparks, are the elementary events in excitation contraction coupling. We have extended our previous Ca^{2+} spark studies to a 3D spatial model of two adjacent sarcomeres. As before, Ca^{2+} spark dynamics in the model depend on myoplasmic and sarcoplasmic buffers, Ca^{2+} uptake by the SERCA pumps and Ca^{2+} diffusion in myoplasm and sarcoplasmic reticulum. Our model has been able to reproduce calcium sparks with a width (FWHM) of $\sim 2\mu$m, as observed in experiments under physiological conditions. We further investigated the effects of Ca^{2+} release current (CRC) amplitude on Ca^{2+} spark width. Our simulations show that for CRC between 4pA-16pA the spark width decreases slowly as current increases. These results predict that as CRC increases, the spark duration decreases which reduces the spark width. Another interesting finding of the model is simulations of Ca^{2+} blinks. Consistent with the recent experimental results, our simulated Ca^{2+} blinks have a FWHM of 0.6 μm. Moreover, the model predicts that experiments underestimate junctional SR depletion because experimental measurement includes Ca^{2+} in the adjacent network SR which is nearly full. The proximity of the SRs makes it difficult to resolve them individually by conventional imaging techniques.

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P-84

Modelling and simulation of polycomb-dependent chromosomal interactions in drosophila

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Gene silencing is a highly complex area of research. Several mechanisms have been identified that inhibit gene expression within the nucleus. It has been observed that silencing via Polycomb Group (PcG) and PcG response elements (PRE) is intensified by the existence of two similar PREs in the nucleus. The two PREs may but do not have to be on the same chromosome. Long-distance pairing between these two loci, which brings them closer together than they would usually be, leads to strong repression of the genes they control.
Our goal is to model and test these geometrical predictions. Within the model we have simulated the movement of the chromosomes in the nucleus. We calculated the expected distance distribution of the two loci in question and compare to experimental results from experiments on drosophila. This comparison gives an estimate on how strong the PcG protein-mediated attraction must be to account for the pairing.

P-85

Dynamics of polymer translocation through a hole

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We study the translocation dynamics of a polymer chain through a narrow hole on the level of scaling law. Particular emphases are put on the role of hydrodynamic interactions and polymer concentration difference between two sides (*cis* and *trans*) of the hole. We show that the presence of the wall has a pronounced effect for the translocation dynamics by controlling the dissipation mechanism for the solution up to some threshold volume fraction. On the contrary, the presence of the wall has only minor effect for a chain moving with Rouse dynamics even in a very dilute solution. When the chain concentration in *cis* side is raised above overlapping concentration, the translocation is driven by the osmotic pressure. This osmotic driven translocation is classified into two regimes depending on the dissipation mechanisms. In each regime, the translocation time is calculated as a function of the chain length and the chain concentration in *cis* side. The effect of the gate size is also discussed.

P-86

Models for studying peptide-lipid interactions

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We have designed a peptide template that inserts into synthetic membranes at low peptide:lipid ratios to form pores that are at least 1 nm in diameter.[1,2] The peptide template is backbone cyclic and symmetrical, permitting facile synthesis by solid-phase methodologies and providing a means for probing the structural parameters governing pore assembly. We are currently using AFM, STEM and dichroism approaches in order to ascertain their key structural features (particularly pore size and peptide pitch), in order to obtain parameters for molecular mechanics calculations. In order to refine the parameters used for these calculations, a simple model system for probing the binding energetics of amino acids with lipid headgroups has been developed.[2,3]
New approaches are being developed (particularly Raman tweezing[4] and Scanning Electrochemical Microscopy) to study the process of peptide insertion into the membrane. Ultimately we will examine membrane activity and pore assembly as functions of peptide structure and membrane composition.
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P-87

On the relation between fluctuation and response in biological systems

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A relationship between fluctuation and response in a biological system is presented. The fluctuation is given by the variance of some quantity, whereas the response is given as the average change of that quantity for a given parameter change. We propose a relationship where the two are proportional, in a similar way to the fluctuation-dissipation theorem in physics. By studying an evolution experiment where fluorescence of protein in bacteria increase, we confirm our relation by observing a positive correlation between the speed of fluorescence evolution and the phenotypic fluctuation of the fluorescence over clone bacteria. The experimental verification will be mainly presented in detail.

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P-88

Opening of sodium channels from a silicon chip – a feasible approach for neural stimulation?

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We investigate non-invasive stimulation of a single neuron via capacitive coupling with a silicon chip. The primary targets for stimulation are voltage-gated sodium channels in the cell membrane.
A proof of principle experiment is performed within a simple model system consisting of $Na_V1.4$ sodium channels in HEK293 cells cultured on a silicon chip. A decaying voltage ramp applied to the chip forces a capacitive replacement current over the oxide and gives rise to a stationary extracellular negative voltage in the cell-chip-junction. The opening of voltage-gated sodium channels located in the attached cell membrane is monitored by a patch clamp amplifier in voltage clamp mode.
The feasibility of this method for neural stimulation under physiological conditions is investigated in experiments with neurons from *Lymnaea stagnalis*. Preliminary results are presented and compared with numerical modelling studies.

P-89

Usage of catalase catalytic active fragments as the new decision in biosensors creation

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We propose an electrochemical model of biosensor for potentiometric definitions of hydrogen peroxide in a water solution consisting in an electrode on the base of catalytic active fragment of catalase and comparison electrode (Ag/AgCl/Cl^-).
Catalase from Sigma C10 having an activity of approximately 2500 IU/mg of protein was used. For reception of the active centre of enzyme including prosthetic group (working element), enzyme was immobilized on different carriers in 0.1 M KH_2PO4 pH 7.0, then was exposed to 0,025% tripsin in 0,2 M Tris-HCl pH 8.0, washed with distilled water and dried on a filter paper at room temperature. An aluminum foil was used as an electrode. Working element was combined with electrode by glues ("Sista", "Pattex", "PVA") or by 7.5 % polyacrylamide gel. The biosensors were exposed to $7x10^{-5}$- $2x10^{-2}$ M H_2O_2. Biosensors with the usage of diasorb DEAE and Al_2O_3, and glue "Pattex" or 7.5 % polyacrylamide gel were more effective. Threshold of sensitivity was $1x10^{-4}$ M. The biosensors should been used for multiple utilization. Life time of the biosensors for definition of low and high concentrations of H_2O_2 is approximately 7 days and activity falls down from 20% to 70% respectively.

P-90

Internal Motility in Stiffening Actin-Myosin Networks

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Eukaryotic cells show an amazing versatility in their mechanical properties. How this behaviour is achieved is matter of multiple interactions between motor proteins, cytoskeletal filaments and regulatory agents. Useful hints to understand possible pathways of such complex organisation can come from studies on simplified networks. Here, we present a study on filamentous actin solutions containing heavy meromyosin sub-fragments of myosin$\sim$II motor molecules. We focus on the viscoelastic phase behavior and the internal dynamics of such networks during ATP depletion by combining micro-rheology and fluorescence microscopy. We observe a sol-gel transition accompanied by a sudden onset of directed filament motion. We interpret such phenomena in terms of myosin$\sim$II enzymology, and suggest a ``zipping" mechanism to explain the filament dynamics.

P-91

Aggregation of meso-tetraphenylporphin sulfonates in the presence of protein

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Self-assembled molecular aggregates are promising materials for applications in many biotechnological fields. One of the most interesting types of molecular aggregates is represented by J-aggregates possessing unique spatial structure and non-linear optical properties. Meso-tetraphenylporphin sulfonates (TPPSn) form J-aggregates spontaneously in acid medium due to interaction of residual negatively charged sulfonic (SO_3^-) groups with protonated central nitrogen atoms. The structure and optical properties of J-aggregates change with the number and position of sulfonic groups. In present work the influence of serum albumin on TPPSn (n = 2, 4) J-aggregates formation in aqueous acid medium was studied by means of absorption and fluorescence spectroscopy. The presence of protein significantly increased the formation of J-aggregates in TPPS4 solution, however had a negligible effect on TPPS2 J-aggregates formation. Dynamic equilibrium between TPPS4 aggregates and monomers was dependent on the molar ratio between porphyrin and protein. The role of different TPPSn-protein interaction in the formation of J-aggregates was discussed.

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P-92

Molecular dynamics simulations of the interaction of cholesterol with DPPC and DOPC monolayers

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We performed molecular dynamics simulations of DPPC and DOPC monolayer as a simple model of lung surfactant monolayer to understand distribution of cholesterol inside the film. Lung surfactant is a mixture of lipids, the majority of which is DPPC and DOPC, that lines epithelial cells of mammalian lungs and plays an important role in stabilizing respiratory mechanism against collapse. We have shown experimentally, using captive bubble surfactometry, fluorescence and scanning probe microscopy that physiological concentrations of cholesterol up to 20wt% does not affect the efficiency of BLES surfactant performance. Here we present 30 ns simulations of DPPC and DOPC monolayer with 0, 5, 10 and 20wt% of cholesterol to understand the effect of cholesterol on the lipid monolayer. Electron density profiles, molecular surface area, tilt angles and chain order parameters were analyzed and compared to data of simulations performed earlier on DPPC and DOPC bilayer. Data of simulation correlated well with experimental results we obtained for BLES surfactant with the presence of cholesterol.

P-93

Spatio-temporal dynamics of synchronous firing in rat cortical neuronal network culture

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We experimentally investigate the synchronous firing dynamics of rat cortical neuronal network culture. The culture forms large clusters (more than 200 micro-meter in diameter) under confluent condition (> 2000 cell/mm^2). The morphology of the clusters varies due to the boundary condition of the culture dish. The culture is fluorescent labeled with Ca ion indicator and detected using fluorescence microscopy. Under depletion of Mg ion, the culture exhibits synchronous firing activity, similar to the epileptic activity in pathology. We found unique spatio-temporal synchronous firing pattern during the maturation process of the culture. In the early stage, the interconnected clusters show delay during synchronous firing due to the spatial heterogeneity of network. The network exhibits more synchronous activity in the maturation stage, with multiple time scale modulation. We explore the spatio-temporal pattern by measuring the correlation function of the firing activity. It shows that there is a predator-prey type non-linear competition in the synchronous firing activity, depicting a global depletion in the necessary ion supply.

P-94

Stochastic dynamics of coupled repressilators

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Background/aim: Intrinsic noise in the gene expression is unavoidable due to a small number nature of biomolecules in a cell. The systematic analyses of the stochastic dynamics, therefore, are most desired to understand the design principle of the gene network. Here, we theoretically examine collective motions of coupled genetic oscillators to examine how the noise affects the oscillatory behavior of those circuits.
Model and Method: Repressilator is a network composed of three genes in which protein synthesized by one gene represses the expression of the other gene cyclically. In usual experimental setup multiple copies of plasmids are embedded in a cell, so that multiple repressilators work in a coupled manner. We perform the stochastic simulation of coupled repressilators with the Gillespie algorithm.
Results: Owing to the noisiness of dynamics, repressilators show the coherent oscillatory behavior only in the limited range of the rate of the gene switching. The range is enlarged and the oscillation is stabilized when the number of coupled repressilators increases. Each of those coupled repressilators, however, does not necessarily oscillate in the same way but there is a fluctuation in the number of repressilators which synchronize with the collective oscillation. This fluctuation leads to the amplitude fluctuation in the number of proteins to show the ordering-disordering transformation in the phase-amplitude space.

P-95

Azimuth-sensitive neurons in the primary auditory cortex of the mouse

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Auditory scene analysis involves identifying the content and location of sounds. An intact auditory cortex is essential for sound localization. Many 'high directional' neurons in the primary auditory cortex of cat and monkey respond strongly to sounds presented across areas as large as half the sound field. No evidence of a space map containing sharply tuned neurons was found in the mammalian auditory cortex.
We found azimuth-sensitive neurons in the primary auditory cortex of mouse. The azimuth sensitivity curves, best azimuth and the preferred range of azimuths (PAR) are determined for individual neurons. The azimuth sensitivity curves are diverse. A majority of neurons have sharp-tuned azimuth sensitivity curves with a narrow PAR from 15° to about 40° at over 20 dB above the threshold. The best azimuths of most neurons were at contralateral 30°, 60° or 90°. The cortical neurons isolated within an orthogonally penetrated electrode have similar azimuth-tuning curves. The neurons in superficial layers of the columns have narrower PARs than those in deeper layers.
In conclusion, it is an evidence for columnar organization of the auditory cortical neurons sharing common functional properties, such as firing patterns, best frequency, minimum threshold, frequency tuning curves, and azimuth sensitivity as well.

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– Protein Reactivity and Dynamics –

P-97

High resolution 1H magic-angle spinning NMR studies of fibrous proteins in the gel state

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A certain number of fibre forming proteins can be prepared in a gel state which is characterized macroscopically by a high viscosity, intermediate between that of a liquid and that of a solid. In this work, we show that this particular state leads to sharp lines in ^{1}H NMR spectra under magic-angle spinning conditions and thus offers the possibility of using high resolution NMR techniques. This effect is evidenced in different systems such as the amyloid model peptide *ccpep* and silk. In order to understand this surprising result, we use both solid- and solution-state NMR techniques to further characterize the conditions in which this effect takes place and the possible internal motions which could be responsible for the partial averaging of anisotropic NMR interactions.

P-99

Dynamics of hydration water and internal motions in proteins

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It is well known that water plays a major role in the stability and catalytic function of proteins. Both the effect of hydration water on the dynamics of proteins and the effect of proteins on the dynamics of water have been studied using inelastic neutron scattering, at the Laboratoire Léon Brillouin. Neutron scattering is the most direct probe of protein dynamics on the picosecond-nanosecond timescale. Results relative to a photosynthetic globular protein, the C-phycocyanin, obtained in protonated and deuterated forms are presented. Molecular dynamics simulation and analytical theory have been combined with neutron data to get a detailed description of diffusive motions, respectively for hydration water and protein. The simulation-derived dynamic structure factors are in good agreement with experiment. The dynamical parameters are shown to present a smooth variation with distance from the core of the protein. The collective dynamics has been investigated using the fully deuterated C-phycocyanin protein. Both the experimental and calculated spectra exhibit a dynamic relaxation with a characteristic time of about 10 picoseconds. Finally, the retardation of hydration water motions is discussed in light of some model of alpha relaxation familiar in the theory of kinetic glass transition in dense supercooled liquids.

P-100

What makes elastase specific?

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The protein responsible for the degradation of *Shigella* virulence factors, neutrophil elastase (NE), is found in granules also containing cathepsin G (CG). Interestingly, although X-ray crystallography reveals that the structures of the inhibited proteins are nearly identical they have different biology; only NE specifically destroys *Shigella* virulence factors. Given that biological molecules are flexible and soft, small changes within the molecular structure may play a significant role in protein function.
To test the hypothesis that the conformations of these two proteins, while active and in solution, are in reality different, we have performed an experiment comparing NE and CG using small angle neutron scattering (SANS). The use of SANS in such a study is possible because, although we measured non-physiological concentration, i.e. 10 mg/ml, enzymatic assay results show that both proteins are still active, at this concentration, and after 72 hours in the beam. Here we discuss on our SANS spectra measured on the V4 instrument at HMI using an incident wavelength of 6Å and three sample detector distances of 12, 5 and 1m covering a Q-range between 0.004 and 0.35 $Å^{-1}$. Although the SANS data did not provide for deriving a unique model, it allowed us to infer that the enzymes possess different substrate specificities because their native conformations in solution are different.

P-101

Destabilization of model membranes induced by Surfactin: dynamics and structural NMR studies

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Surfactin (SF) is a lipopeptide secreted by *Bacillus subtilis*. Composed of a peptidic backbone (7 a.a.) completed by an aliphatic chain (12-14 C atoms), it was named after its surfactant abilities. Its biological properties are interesting as well: SF is a strong hemolytic, antimycoplasmal and antimicrobial lipopeptide that induces a certain destabilization of biomembranes.
Membrane models representative of mycloplasmas (DMPG($^2H_{54}$)/SM) and erythrocytes (DMPE/DMPC($^2H_{54}$) were used to study the effect of surfactin. The basic DMPC($^2H_{54}$) model membrane was also used.
Membrane dynamics are studied by solid-state ^{31}P, ^{2}H and ^{14}N NMR. We have shown that SF induces a light destabilization of DMPC membrane, but has a critical effect on mycoplasmas-like membrane (powder spectrum is evolving to isotropic peak). Next studies will be done on erythrocytes-like membrane.
In order to understand more precisely SF-lipids interactions, structural studies of SF are also made. Based on ^{1}H liquid experiments (TOCSY, NOESY…), we got the 3D structure of SF in organic solvent. We are now working on getting SF structure when inserted in lipid bilayer using HR-MAS NMR.

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– Protein Reactivity and Dynamics –

P-102

Structure and function of DNA-binding proteins in thermoacidophilic archaea Sulfolobus

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The forming of multiprotein-DNA complexes is an essential process during the life cycle of the cell, including the transcription, recombination, and replication pathway. To elucidate the mechanism of recognition and regulation performed by these proteins, several interesting proteins were chosen from thermoacidophilic archaea *Sulfolobus*. Then their three-dimensional structures will be determined by X-ray crystallography or NMR methods. In our preliminary result, two structures of small chromosomal binding proteins, Sac7d/Sso7d and Sso10b2 were solved, and the DNA-bending property of Sac7d/Sso7d has been well studied. The recombinase RadA, which has the ability of self-polymerization, is characterized in the repair of DNA. A repressor-like regulatory protein Sso7c4, and the apoptosis related protein Pdcd5 were also analyzed. The interaction of these proteins with their DNA targets can provide us an integral view of the variety of binding specificity and then to apply the modulation to the DNA activity.

P-103

Step-by-step mechanisms of gaseous anaesthetics

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Anaesthesia states from early stages, such as amnesia and hypnosis, to deep sedation and lack of responses to noxious stimuli are thought to be mediated by separate mechanisms, though this has not been proven. We propose here a step-by-step mechanism. Inhaled anaesthetics would first bind to large hydrophobic cavities within brain cytosolic proteins, disrupting their function to produce early stages of anaesthesia. Then, when body gas concentration raises with the cytosolic protein binding sites fully occupied, inhaled anaesthetics would further bind to small hydrophobic cavities within ion-channel receptors, leading to surgical anaesthesia. Xenon and nitrous oxide binding abilities within two proteins, urate oxidase, a prototype of a cytosolic protein, and annexin V, which can be considered as a prototype for ion channels such as the NMDA receptor, have been investigated by X-ray crystallography under gas pressure. The ratio of volume expansions of the gas binding site cavities is correlated to the ratio of anaesthetic potency assessed with *in viv*o physiopharmacology, suggesting this step-by-step mechanism of anaesthesia.

P-104

X-ray produced metastable Hb state relaxes within the same quaternary R structure

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Hemoglobin (Hb) is characterized by a quaternary low affinity T state and high affinity R state when all its four subunits are unligated or ligated respectively. O_2 binding to heme Fe(II) leads to the T – R transition through a quaternary structural change. Methemoglobin (Hbmet) where iron(III) coordinates water at the 6^{th} site is in the R conformation. The Hb conformational changes have been studied following the relaxation of its metastable Fe(II) low spin state produced with X-ray irradiation of Hbmet samples at 100K. The relaxation of the metastable state at 160K, 170K, 180K and 190K has been fitted with energy barrier distributions. The enthalpy distribution becomes temperature dependent at 180K, this is the characteristic temperature of the dramatic enhancement of the protein flexibility. In this state the protein can fluctuate among different conformational structures. Therefore two processes are present: a structural relaxation of the metastable state to a new equilibrium state and a structural fluctuation within the same conformation. This work deeps the correlation between these two phenomena. Comparing the relaxation of the metastable state for hemoglobin and myoglobin shows a stabilizing effect of R quaternary structure.

P-105

Effect of lipid oxidation on factor XIII transglutaminase activity; infrared structural studies

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Transglutaminases catalyze irreversible intermolecular cross-link between proteins producing high-molecular-mass aggregates stabilized by isopeptide bonds. The enzyme is implicated in multiple in important cellular phenomena. It has been suggested that phospholipids can play a role in the regulation of the enzyme and oxidation can enhance the activity. We have studied the biochemical and structural changes induced in factor XIII transglutaminase, involved on blood clotting after oxidation in the presence of lipids and the effect of antioxidants on the enzyme activation. The secondary structures of factor XIII in soluble form and in the presence of PC large unilamellar vesicles (LUVs) are very similar. However, after LUVs oxidation there is a change in the structure involving an increase in â-sheet structure. This structural change is associated with enzyme activity. Thus, the presence of non-oxidized LUVs does not affect enzyme activity whereas oxidized lipid increases enzyme activity. The amount of activation is dependent on the lipid:protein ratio. The presence of vitamin E prevents the increase in activity produced by oxidation.

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– Protein Reactivity and Dynamics –

P-106

HIV-1 Integrase: study of 3´-processing reaction by steady-state and pulsed fluorescence anisotropy

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Integrase (IN) of the Human Immunodeficiency Virus (HIV-1) is the enzyme responsible for the concerted integration of the viral DNA into the host DNA. The overall integration process is a two-step process. In the first chemical step, a dinucleotide is cleaved from the 3 'ends of the viral DNA. The newly exposed hydroxyl groups are used in the second chemical step to attack phosphodiester bonds on opposite strands of the target DNA, joining the 3' viral DNA ends to the target DNA. Here, we proposed an integrase assay based on steady state fluorescence anisotropy, using fluoresceine-labeled oligonucleotides that mimic one extremity of viral DNA. This real-time assay is useful to monitor simultaneously both the integrase-DNA interactions and the subsequent kinetic of the 3'-processing activity. Applications for studying the mechanism of action of IN inhibitors are described. The anisotropy-based assay is also suitable for screening approaches using 96-well plates. Finally, steady state anisotropy as well as time-resolved fluorescence anisotropy indicates that high-order multimeric forms of IN are detrimental for the 3'-processing activity.

P-107

Lipid induced conformation of the tachykinin neuropeptides Scyliorhinin I and Uperolein

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The mammalian tachykinin (TK) peptides and their three Neurokinin (NK1, NK2 and NK3) receptors represent an effector system with pathophysiological relevance in various diseases including asthma, emesis and depression. The promiscuous tachykinin -NK receptor interactions and incompletely overlapping functions mediated by each NK receptor may indicate added therapeutic benefit of using multiple NK receptor blockade. Scyliorhinin I, a linear decapeptide, is the only known tachykinin that shows high affinity for both NK1 and NK2 binding sites and hence is a promising tool in tachykinin receptor family. Similarly Uperolein, a physalamine-like endecapeptide, is the potent NK1 receptor agonist. As a first step to understand the structure-activity relationship of these two novel peptides, we have investigated their membrane-induced conformation using techniques of circular dichroism and two-dimensional NMR spectroscopy. Sequence specific resonance assignments have been made from correlation spectroscopy and NOESY and the family of structures have been calculated using Torsion Angle dynamics algorithm DYANA. Analysis of NMR data is suggestive of the presence of 3_{10}-helix that is in equilibrium with an α helix from residues 4-10 in Scyliorhinin I and from residue 5-11 in Uperolein preceded by turn at N-terminus. An attempt has been made to correlate the observed conformational differences to the binding ability and biological activity of various Neurokinin receptor agonists.

P-108

Protein-water displacement distributions

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The statistical properties of fast protein-water motions are analysed by dynamic neutron scattering experiments. Using isotopic exchange one probes either protein- or water hydrogen displacements. A moment analysis of the scattering function in the time domain yields model-independent information such as time-resolved mean square displacements and the Gauss-deviation. From the moments one can reconstruct the displacement distribution. Hydration water displays two dynamical components, related to librational motions and anomalous diffusion along the protein surface. Rotational transitions of sidechains, in particular of methyl groups, persist in the dehydrated and in the solvent-vitrified protein structure. The interaction with water induces further continuous protein motions on a small scale. Water acts as a plasticizier of displacements, which couple to functional processes such as open-closed transitions and ligand exchange.

P-109

Reduced global cooperativity is a common feature underlying the amyloidogenicity of lysozyme mutations

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Four single-point mutants of human lysozyme (I56T, F57I, W64R and D67H) are associated with a systemic amyloid disease. We have used a variety of techniques including H/D exchange experiments monitored by NMR and mass spectrometry to study the effects of the I56T and D67H mutations on the stability and dynamics of the protein. These experiments show that, despite their different position in the sequence and their different effect on the native state of the protein, both mutations significantly reduce the stability of the β-domain and the adjacent C-helix in the native structure allowing the formation of a cooperatively partially unfolded state even under physiologically relevant conditions. The effects of the binding of a camelid antibody fragment (cAb-HuL6) on the properties of the two variant proteins were probed. These experiments indicate that the binding of cAb-HuL6 significantly stabilizes those regions of the protein involved in the locally cooperative unfolding event. The result of this binding is to prevent the ready conversion of the variants into their aggregated states. These data, combined with NMR and X-ray structural analyses of the complex, suggest a mechanism by which cAb-HuL6 stabilises the native state of the variants of lysozyme. Taken together these findings contribute to a better understanding of the mechanism of amyloid formation.

Posters
– Protein Reactivity and Dynamics –

P-110

Solvation, stability and solubility of halophilic proteins

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Halophilic proteins have evolved specific molecular mechanisms – acidic residues; multiple salt bridge between sub-units- that allow them to be both stable and soluble in the high KCl concentration of the cytoplasm of extreme halophilic microorganisms. We evaluated the solvation, stability and weak inter-particle interactions of malate dehydrogenase from *H. marismortui* (*hM* MalDH) in various salt solutions, in order to probe the role of the ions and water of the solvent. *hM* MalDH adapts to its environment: its global solvation depends strongly on the salt nature. Strong (detected by crystallography) and weak binding sites for solvent ions explain the effect of salt on protein stability and auto-association. Attraction between proteins is observed when the composition of the solvation shell is different from the bulk, which can be understood by thermodynamic relationships. Halophilic proteins have adapted their interactions to develop interactions with salt that avoid water enrichment to remain soluble. These studies explain the adaptation of halophilic proteins and also their protocols of crystallization.

P-111

Calcium-dependent interaction of annexin A1 with solid-supported membranes

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The binding of annexin A1 to solid supported lipid bilayers consisting of POPC/POPS (4:1) mimicking the cell membrane has been investigated as a function of calcium concentration of the bulk phase. Quartz crystal microbalance measurements in conjunction with fluorescence microscopy and computer simulations indicate that annexin A1 adsorbs preferentially and irreversibly on the POPS enriched phase forming two dimensional protein aggregates. Annexin A1 adsorbed on the POPC-enriched phase, however, desorbs after washing with buffer. The overall area occupied by the POPS-enriched phase can be controlled by the calcium concentration. Monte Carlo simulations suggest that the area of the POPS-enriched phase increases from 4.3 to 24.3 % by changing the calcium concentration from 0.01 to 1 mM.

P-112

Calponin gelsolin interaction

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Gelsolin and calponin are present in the same structures within the cells, namely lamellipodia, and along some stress fibers to different extends, suggesting potential direct or indirect interactions between gelsolin and calponin. Examination of co-localization in Cos cells by immunofluorescence and co-immunoprecipitation experiments suggested for the first time, the occurrence of a direct relationship between these two actin binding proteins. Gelsolin calponin complex formation was then tested in vitro by fluorescence and affinity chromatography approaches. Fluorescence experiments clearly demonstrated the interaction of gelsolin with two calponin isoforms (basic h1 and acidic h3) with high affinity. In addition, this binding is calcium and pH dependent, indicating a possible regulation by the conformational state of gelsolin molecule. In fact, the interface between these two proteins needs the participation of the severing and the regulatory domains of gelsolin. One of the well-known properties of gelsolin is to greatly enhance the rate of actin polymerization. More interestingly, the presence of calponin partially inhibits this gelsolin activity. Therefore, we suggest that interaction of calponin with gelsolin plays an additional regulatory role in the formation of actin filaments.

P-114

HIV entry process : Interaction between CD4 and CCR5 receptors followed by FRET

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HIV entry relies on sequential interaction between its envelope, CD4 and either CCR5 or CXCR4 in order for the fusion of viral and cell membranes to proceed. Beyond these interactions, numerous studies suggested that organization of receptors at the plasma membrane of host cells is critical for viral entry. In the present study, we analysed by FRET, the in vivo membrane interaction of CD4 and CCR5 receptors, respectively labelled with EBFP and EGFP. These receptors were co-expressed in HEK 293T cells using retroviral transduction system. Then we have established a polyclonal stable cell line with a low expression level. This avoids unspecific FRET due to a large receptor surface density. A polyclonal cell line was used to obtain relevant physiological effect. Then a microspectrofluorimetric approach was imposed with measurements performed cell by cell. To characterise and to classify a huge number of spectra, trichromatic co-ordinate spectra conversion was used. A constitutive interaction of CD4 and CCR5 was found. The perturbation of receptors association was studied by addition of CCR5 agonist (MIP-1β), soluble form of CD4, and gp120 protein (involved in the earlier step of VIH infection). We also investigated the reversible effect of various truncated forms of CD4 on this CD4-CCR5 basal association. We are able to identify subdomains of CD4 involved in the interaction with CCR5.

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– Protein Reactivity and Dynamics –

P-115

Lateral mobility in membranes revisited: a simpler law from lipids to large proteins

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The study of lateral mobility is a key widely used to obtain valuable informations about the properties of membrane proteins incorporated into cells, Vesicles or into bilayers. Most of the studies focus solely on the radius of the diffusing protein, by far the most interesting parameter for biologists. In order to extract that value from the measured diffusion coefficient D, one need to use a hydrodynamic model created 30 years ago by Saffman and Dellbrück : $D_{Saffman} = \frac{kT}{4\pi\mu_m h}\left(\ln\left(\frac{\mu_m h}{\mu_w R}\right) - \gamma\right)$
Our purpose was to weight experimentally the role of each adjustable parameter: the radius R of the diffusing object, the thickness h and the viscosity μ_m of the bilayer and the viscous coupling to the aqueous phase through its viscosity μ_w.
Up to 10 different peptides and proteins were incorporated into a versatile phase of model bilayers, allowing us to modulate at will the bilayer thickness and the viscosity of water.The results obtained in our systems show a strong disagreement with the above relation and are well fitted by a heuristic simple law: $D = \propto \frac{kT}{\mu_m h.R}$
Along our investigation we will emphasize various applications, such as a test to determine the orientation and the anchoring of peptides into membranes. We point attention to the dramatic effects of glycerol or sugars on the measured diffusion.

P-117

Dynamics of a ternary cytokine-receptor complex on model membranes

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The dynamics of lateral interactions within the plane of the membrane plays a key role for transmembrane signalling. We have investigated the recruitment of the two components of the type I interferon (IFN) receptor, ifnar1 and ifnar2, by IFNs on model membranes *in vitro*. The extracellular receptor domains ifnar1-EC and ifnar2-EC were tethered onto solid-supported, fluid lipid bilayers in an oriented manner. Ligand binding and receptor cross-talk was monitored independently by simultaneous detection of total internal reflection fluorescence spectroscopy and reflectometric interference. Wild-type IFNα2 and mutants binding to ifnar1-EC and ifnar2-EC with different rate and affinity constants, were site-specifically labelled with fluorescence dyes. Using ligand binding assays, as well as pulse-chase and FRET experiments, we fully dissected the interaction rate constants of the formation and dissociation of the ternary complex. Thus, we determined for the first time protein interaction rate constants in plane of the membrane. Strikingly, we found lower dissociation rate constants for the proteins tethered on the membrane compared to the interaction in solution, suggesting that the reaction coordinate is affected by restricting diffusion into two dimensions. The lateral association rate constants obtained from these assays indicated that the encounters were much more productive on the membrane than in solution, which could be ascribed to the optimized orientation of the proteins tethered to the lipid bilayer.

P-118

Viscoelastic properties of antibody films

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The Young's dynamic modulus *E* and damping logarithmic decrement ***è*** of fixed amorphous films of IgG class antibodies are measured depending on relative humidity at temperature 25^{o}Ñ by Morozov's micro method. Method is based on the analysis of electrostatically raised cross-section resonant fluctuations of console fixed plates [1]. This method allows investigating microsamples with thickness up to 10 microns, width 100 microns and length up to 1 mm. Also hydration isotherms of fixed and nonfixed films of antibodies are measured by the method described in [2]. The influence of fixing by glutar aldehyde and process of ageing films on an isotherm of hydration are investigated. It is shown, that the degree of fixing strongly influences characteristic behavior of the films. Films of antibodies are characterized by smaller value of the Young's modulus in comparison with films and crystals of globular proteins. Character of dependence *Å* and ***è*** from humidity has complex nonmonotonic character that is impossible to explain only by change of the water content in films.
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How elastic are biopolymers? Mechanical properties of proteins

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The viscoelastic properties of solid samples (crystals, amorphous films) of hen egg white lysozyme, bovine serum albumin, and sperm whale myoglobin were studied in the temperature range of 100-300^{o}K at different hydration levels. Decreasing the temperature was shown to cause a steplike increase in the Young's modulus of highly hydrated protein samples (with water content exceeding 0.3 g/g dry weight of protein) in the temperature range of 237-251^{o}K, which we refer to as a mechanical glass transition.
Soaking the samples in 50% glycerol solution completely removed the steplike transition without significantly affecting the glass transition. The apparent activation energy determined from the frequency dependence of the glass-transition temperature was found to be 18 kcal/mol for wet lysozyme crystals. Lowering the humidity causes both the change of the Young's modulus in response to the transition and the activation energy to decrease. The thermal expansion coefficient of amorphous protein films also indicates the glass transition at 150-170^{o}K. The data presented suggest that the glass transition in hydrated samples is located in the surface layer of proteins and related to the immobilization of the protein groups and strongly bound water. The viscoelastic properties of globular proteins were found to display marked heterogeneity resulting both from its domain structure and enhanced mobility of surface layer.

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Kinetics and thermostability properties of guaiacol peroxidase in dormant Crocus sativus L. corm

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Peroxidases are metabolizing heme enzymes widely distributed in plants and animals and involved in important physiological roles. Plant peroxidases, including guaiacol peroxidase, have been associated with a number of functions from root elongation to oxidative stress protection. *Crocus sativus* L. (hereafter saffron), the plant that produces saffron, is propagated only via its corm. In this work, guaiacol peroxidase activity was investigated in dormant saffron corms. Crude extract prepared from dormant saffron corms exhibited guaiacol peroxidase activity that was optimum at pH 7.5. At optimum pH, the enzymatic activity was characterized by a Km of 10.5 mM for guaiacol, a Vmax of 51 nmol/min/mg prot, and a catalytic efficiency of 0.0016 per min and per mg prot. It was inhibited by cyanide, azide and ascorbate with minimum inhibitory concentrations of 0.165, 0.17 and 0.016 mM, respectively. Thermostability studies were conducted by incubating aliquots of corm extract at various temperatures (25°C-70°C) for 15 min, then either at 0°C for 15 min or at room temperature for 15 min. Enzymatic activity was then immediately assayed at room temperature, at pH 7.5. Results showed that under both experimental conditions the activity was stable at up to 55°C and degraded rapidly with just a slight increase in temperature above 55°C; the transition temperature was $\sim$ 57°C as given by Arrhenius plot.

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On relation between adriamcyn cardiotoxicity and trace elements, blood parameters and free radicals

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Trace elements in organism carry out various important biological roles by forming complexes with enzymes. Present study was planned to determine the possible cardiotoxic effects of dox. On the other hand, in Hct, MCV, MCH, RDW value's decrease was detected only in male animals of experimental group. Copper and zinc levels in liver and heart tissues of experimental males were found higher than the ones in control group. While increased protein oxidation was detected in heart tissue of experimental females ($p<0,05$), decreased protein oxidation was found in experimental males ($p<0,05$). Also, increased protein oxidation was determined in liver tissue of experimental males ($p<0,01$). As results of present study it can be concluded that: performing only MDA measurement is not sufficient enough, because nonsensed tissue oxidations may arise. So protein oxidation also has to be measured to see the oxidative effects of Dox. Not only the effects of Dox on blood parameters and the degree of oxidative stress in tissues is dependent to the age, but also it varies according to the sex, it may be said that even in the same sex group Dox shows different oxidative effects in different organs.

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Interaction of ezrin with artificial lipid membranes

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Members of the ezrin/radixin/moesin (ERM) protein family are known to connect the plasma membrane with cytoskeletal components of the cell, thereby determining cell shape and motility. Here, the membrane binding capabilities of ezrin were investigated by means of the quartz crystal microbalance (QCM) technique. The QCM-technique enables one to monitor the adsorption of proteins on solid supported membranes in a label-free and time-resolved manner due to the correspondance of frequency change to adsorbed mass. From the frequency readouts detailed information such as binding constants and adsorption and desorption kinetics can be obtained.
In our setup, one of the two gold electrodes of a 5 MHz AT-cut quartz plate was functionalised with an octanethiol monolayer. Subsequently, a second monolayer composed of POPC doped with PIP_2 (Phosphatidylinositol-4,5-bisphosphate) was physisorbed. The quality of the resulting bilayer was monitored by impedance measurements.
Based on this solid supported membrane system, the binding of ezrin and its N-terminal domain (N-ERMAD) was quantified. In particular, the influence of PIP_2 on the binding affinity and binding kinetics was investigated in detail. Based on the obtained results, further studies will be carried out to elucidate the interaction of ezrin with its dimeric ligand S100P, which is thought to modulate the ezrin-actin-interaction.

P-123

Technical developments in linear dichroism of biological molecules

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In order to understand the function of many biological macromolecules, structural information is invaluable. Here we demonstrate advances in a structural technique, linear dichroism (LD) and the implications of these advances for the study of a wide range of biological systems. For example, LD is suitable for the study of DNA interactions with proteins or small molecules, membrane systems and fibrillar proteins.
LD is the differential absorbtion of light polarised parallel and perpendicular to an orientation axis. Orientation can be achieved in different ways: for example using magnetic fields, stretched films, squeezed gels or shear flow. Our preferred way of orientation is by Couette flow, where the sample is stirred by rotation of a spindle relative to a sleeve. The sample aligns with the long axis of the molecule perpendicular to the spindle axis. The LD of this aligned system can yield information on the orientation of chromophores within the molecular complexes present. Measurement of the strength of interactions, changes in the stiffness of long polymers and the orientation of monomers within polymers and membranes can all be achieved using LD.

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Narrowing of the chromophore spectra by the protein globule of green fluorescence protein

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Green fluorescence protein (GFP) has very rigid chromophore pocket, which isolates the chromophore from the solvent. We used this protein to study the effect of the isolation of a chromophore on its spectral and dynamic properties.
The fluorescence spectra of GFP and the model of its chromophore *p*-Hydroxybenzylidenedimethylimidazolone (*p*-HBDI) in glycerol/water 60/40 v/v solution were obtained in the wide interval of temperatures, 80 – 300 K. The protein spectra consist of a progression of well resolved narrow bands, which broaden and shift to the red upon the heating. The model compound spectra are very wide and have no structure at all temperatures.
The linear red shift of the protein spectra points to the presence of the chromophore coordinate Q, which is quadratically coupled to the electronic transition under consideration. Taking into account of the distribution of the model molecules along this coordinate allowed us to reconstruct the HBDI spectrum at the lowest temperature. These results show that motion along Q (most probably *cis-trans* isomerization) essentially affects the chromophore.

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Highly shifted pKa values of ionizable residues at 25 internal positions of staphylococcal nuclease

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Internal ionizable residues in proteins usually titrate with highly shifted pKa values because they are dehydrated and the protein interior is not as good a solvent as water. The determinants of these pKa shifts are poorly understood. To address this issue, the pKa values of Glu and Lys residues in 25 internal positions of a hyper-stable variant of *staphylococcal* nuclease were measured with equilibrium thermodynamic methods. The pH dependence of stability, of the intrinsic fluorescence, and of the far-UV CD signal were also used to assess the structural consequences of the substitution and ionization of these internal residues.
The median cost of replacing internal positions with the neutral form of the ionizable group was 4.6-kcal/mol for Glu and 3.9-kcal/mol for Lys. Of the 50 variants, 40 titrated with perturbed pKa values. The ΔpKa ranged from 0.5 to 4.9 for Glu residues, and from 1.1 to 6.4 for Lys residues. The median cost of ionizing internal Glu was 4.4-kcal/mol; for internal Lys it was 2.6-kcal/mol. Of the 50 variants studied, 33 seem to be able to tolerate both the substitution and the ionization of the internal group without any detectable change in conformation (16 Glu; 17 Lys). Of the remaining 17 variants, 2 did not tolerate the substitution (2 Glu; 0 Lys), and 15 tolerated the substitution but locally (6 Glu; 5 Lys) or globally (1 Glu; 3 Lys) unfolded when the internal groups ionized. Collectively, these data suggest that the protein interior can be considerably less hydrophobic than commonly assumed.

P-126

Interactions of hemoglobin with selected anesthetics: a VCD study

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Currently there is no completely accepted theory about the mechanism of general anesthetics. It is believed that these interact either with membranes altering their physical structure, or directly with proteins either via specific binding interaction or unfolding of the proteins to a dysfunctional state. In this current study, the interactions of two general anesthetics with hemoglobin were investigated by vibrational circular dichroism (VCD) spectroscopy. This method is a relatively new chiraloptical technique, which has proven to be particularly advantageous for investigating the secondary structure of proteins and DNA. In this study VCD revealed a change in the secondary structure of hemoglobin upon addition of the two general anesthetics ethanol and halothane. The observed change appeared to be concentration as well as time dependant. Both ethanol and halothane demonstrated a specific concentration range in which the secondary structure of the hemoglobin is altered to a maximal extent from a mainly α-helical protein to one that contains a large amount of β-sheet content.

P-127

Towards atomic detail of human nuclear lamin assembly

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Intermediate filaments (IFs) are principal components of the cytoskeleton in higher eukaryotic cells. The molecular architecture of IFs, i.e. the specific way the elementary IF dimers assemble into a filament, is currently only poorly known. Lamins form a special class of IF proteins found in cell nucleus. The first step in lamin assembly is the interaction of the N- and C-terminal regions of the elementary dimer. While crystallisation of the full-length lamin dimer is problematic, we have generated a series of shorter lamin fragments suitable for obtaining three-dimensional crystals. The formation of stable heterotetramers by pairs of N- and C-terminal fragments was investigated by analytical ultracentrifugation and circular dichroism spectroscopy. The N-terminal fragments from lamins A and B1 form stable dimers. Furthermore, when the N- and C-terminal fragments were mixed at 1:1 ratio, heterotetramers were detected. The ultimate goal of this project is to elucidate the three-dimensional structures of individual lamin fragments and the structure of the N-C overlap of the dimers. This information is of great importance for the understanding of IF function in the living cell as well as of the mechanism of various human diseases linked to mutations in IF proteins.

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Heme pocket dynamics of heme proteins: combined quantum chemical, vibronic and spectroscopic study

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The combined experimental and theoretical study of the relationship between the structure, dynamics and function of heme proteins and its dependence on the solvent is presented.
The infrared absorption CO band of the carbonyl horseradish peroxidase (HRP(CO)) with and without benzohydroxamic acid in glycerol/water and trehalose at pH 6.0 and 9.3 was studied at different temperatures, 10 – 300 K. These spectra and the temperature dependence of the CO band of myoglobin (Mb(CO)) and hemoglobin (Hb(CO)) were analyzed by using theory of optical absorption bandshape and the results of the study (quantum chemical calculations and vibronic theory of activation) of the effect of the external electric field on the C-O frequency.
Heating of the samples to the room temperature leads to the entrance of a water molecule in the spacious heme pocket (HRP(CO) and "open" conformations of Mb(CO) and Hb(CO)), causing a transition of the protein into new conformational substate. The entrance of the water molecule is expected to affect the protein function.
These conclusions are applicable to other proteins with large hydrophobic pockets.

P-129

Employing long range surface plasmon fluorescence spectroscopy in biosensors

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Surface plasmon resonance has wide applications in the field of spectroscopy and surface plasmon fluorescence spectroscopy, since been used in biosensors, has become a central tool for quantifying biomolecular interactions. In the few studies done before about long range surface plasmon resonance, it has not been used in fluorescence spectroscopy. In this work, we demonstrate that LRSPR, if being combined with fluorescence spectroscopy, could be an effective tool for detecting small molecules with small concentrations using the field enhancement at the interface which is higher than the conventional SPR. Also some fundamentals of LRSPFS were studied leading to figuring out some interesting differences between LRSPFS and SPFS.

P-130

Structures and dynamics of peptides involved in cancer by solid-state NMR and circular dichroism

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Growth factor receptors of the tyrosine kinase family consist of two large, separately folded domains, one external and one cytosolic, connected by a single transmembrane segment. The sequence of the membrane spanning peptides appears to be important for function. For example, a single point mutation in the proto-oncogene c-erbB2/neu, resulting in a substitution of a valine residue to glutamic acid at position 664 within the transmembrane region, may transform it into an oncogene.
Solid phase synthesis of Neu (Val664) and Neu* (Glu664), the 35 amino-acid domain has been accomplished. The use of peculiar purification and cleavage conditions provided high yield. Peptide purity is more than 96% and was controlled by mass spectrometry.
The peptide can be solubilized in TFE or incorporated into bicelle membranes. In those media dichroism circular demonstrated that peptides adopt a helical structure. Our results show that the flat membrane is necessary for the oligomerization peptides.
Peptides are incorporated into liposomes and we used quadrupolar and chemical shielding NMR interactions (^{31}P, ^{2}H and ^{14}N) to determine alterations of internal membrane dynamics.

P-131

Crystal structure of a bacterial immunity protein PedB conferring immunity to pediocin PP-1

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Bacteriocins produced by lactic acid bacteria are ribosomally synthesized antimicrobial peptides. Among them, pediocin-like bacteriocins (Type IIa bacteriocins) constitute an important and well-studied class of antimicrobial peptides. In general, genes encoding pediocin-like bacteriocins are cotranscribed with, or in close vicinity to, a gene encoding a cognate immunity protein which protects the bacteriocin-producer from their own bacteriocin. We report here the crystal structure of 112-amino acid immunity protein (PedB) for Pediocin PP-1 from *Pediococcus pentosaceus*. The crystal structure of PedB revealed that it consists of an antiparallel 4-helix bundle. The fact that the immunity proteins conferring immunity to carnobacteriocin B2 and enterocin A, which are members of the Type IIa bacteriocin, also consists of a 4-helix bundle strongly indicates that this is a conserved structural motif in all pediocin-like immunity proteins.
The C-terminal ends of immunity proteins against pediocin-like bacteriocins are thought to be flexible and be important for the recognition of the cognate bacteriocins. Consistently, the C-terminal end of PedB (residues 94-112) is disordered, suggesting that this region may be also flexible.

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– Protein Reactivity and Dynamics –

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Detecting conformational fluctuations of ubiquitin and NEDD8 by the variable pressure NMR

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The variable-pressure NMR technique has been used to investigate the conformational fluctuations of two functionally similar ubiquitin-like proteins (Ubl), ubiquitin and NEDD8. The technique enables one to access higher energy conformers of a protein utilizing the intimate relationship between conformation and partial molar volume. We found that ubiquitin exists as an equilibrium mixture among at least 4 different conformers (N1, N2, I and U), and determined NMR structures at 30 bar and at 3 kbar, giving "NMR snap shots" of a fluctuating ubiquitin structure between N1 and N2. (Kitahara et al. JMB 347, 277-, 2005). Human NEDD8 is an 81-residue Ubl (60 % sequence identity with ubiquitin). Large amplitude fluctuations, estimated in the time range of micro to milli seconds, are found by the NMR spin relaxation analysis for the folded conformer normally designated as "native (N)". Furthermore, a peculiar intermediately folded conformer (designated as "I") is found, which undergoes local unfolding in the entire C-terminal side of the protein. The structure of the conformer (I) is essentially the same with that of the conformer (I) in human ubiquitin, although their equilibrium populations are different. We found that the local unfolding takes place preferentially in regions where the amino acid sequence is conserved between ubiquitin and NEDD8, which strongly suggests that this local unfolding is evolutionally designed for the two proteins to perform their almost common function.

P-133

Inter-subunits region differences in *E. coli* and *P. vulgaris* tryptophanase affect cold lability

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We studied the reversible cold dissociation of tryptophanase (Trpase) from *E. coli* and *P. vulgaris*. HPLC analyses show that the tetrameric apoform of tryptophanase (Trpase) from *E. coli* readily dissociates into dimers (70.4±2.5 %) even at 25^{o}C. Trpase from *P. vulgaris* at 25^{o}C, in addition to dimers, dissociates also to monomers (18.2 ± 4.0 %). Cooling of the *P. vulgaris* enzyme solution to 1^{o}C for 24h, augmented the dissociation into monomers (32.9 ± 1.6%). Thus, in contrast to the *E. coli* apoenzyme, apoTrpase from *P. vulgaris* exists in a dynamic equilibrium between tetramers, dimers and monomers. Reactivation of the apoTrpases by adding pyridoxal phosphate and heating to 37^{o}C 30 min resulted in a full restoration of the tetrameric active form. Superposition of the X-ray crystallography inter-subunit region contacts, which comprises of four antiparallel â-strands; two strands from each subunit compose a network mainly responsible for keeping the dimeric structure, in the *E. coli* and *P. vulgaris* Trpases shows that in both Trpases, these four â-strands are very similar. However, comparison between the amino acid sequences in these strands show that in *E. coli* Trpase there are more hydrophobic amino acids than in *P. vulgaris* Trpase. The role of hydrophobic forces in maintaining the dimeric form of Trpases is discussed.

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Red-edge excitation fluorescence studies of the fast intramolecular dynamics of EMAP II cytokine

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Endothelial monocyte activating polypeptide II (EMAP II) is a novel cytokine which also displays tRNA binding activity. The functional properties of EMAP II depend on conformational rearrangements which may be modulated by protein intramolecular flexibility. We have studied the nanosecond protein dynamics of EMAP II at the temperature range 25°C-50°C. EMAP II contains single Trp125 residue which is an intrinsic fluorescent probe in protein structure. The fluorescence emission of EMAP II Trp125 reveals a maximum position at 335 nm that indicates for a mainly buried state of Trp125. We explore the dynamics of Trp125 microenvironment using the red-edge excitation shift (REES) effects. Fluorescence spectra of EMAP II were measured in the range of the excitation wavelengths 290-304 nm at 25°C and detected a high REES effect (about 10 nm). These data indicated the absence of the fast structural relaxation of Trp125 microenvironment at 25°C and Trp125 shielding in protein. The temperature dependence of EMAP II REES was studied in order to monitor the conformational changes of the protein. It was found that REES effect gradually decreased as the temperature was raised from 25°C to 37°C. This effect was due to the local conformational change of Trp125 microenvironment induced by physiological temperature. It is proposed that the flipping of Trp125 at physiological temperature may be essential for the binding with tRNA during recognition process.

P-135

Effects of hydration and interactions with chemical chaperones on protein dynamics

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Work is devoted to the study of influence of hydration water and of chemical chaperones on the dynamics and reactivity of lysozyme, myoglobin and photosynthetic Reaction Centre (RC). Simultaneous increase of stability and reactivity of enzymes during interactions with chemical chaperones was observed recently. Dynamic and structure changes were investigated by Rayleigh scattering of Moessbauer radiation, diffuse X-ray scattering and by conventional molecular dynamic simulations. Data for hydrated proteins approves the existence of cooperative motions of alpha helices and beta sheets, as well as of individual motions. Hinge bending motions for lysozyme have more complicated character than plain motions of domains. One may make a conclusion that water during hydration competes with intramolecular hydrogen bonds, loosens the protein and increases internal dynamics. Concurrently water arranges the ordering of macromolecules. Difference in architecture is the reason of different dynamics of myoglobin and lysozyme. Cooperativity of motions and ordering in RC is essentially higher than that in Mb and lysozyme. Dynamics of lysozyme strongly correlates with its reactivity when enzyme is interacting with chemical chaperones.

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Induced fit revisited: protein-ligand docking refinement using normal mode amplitude optimisation

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Upon ligand binding, receptors often exhibit significant conformational rearrangements commonly referred to as induced fit. Predicting such changes in receptor structure is an important challenge in order to improve automated docking methods and structure-based drug design. In this work, we propose the use of all-atom normal modes to model structural flexibility during refinement, in particular applied to docking with small ligands. Normal mode analysis in the framework of the Elastic Network Model provides an efficient tool for extracting principal low frequency motions of proteins, and by performing refinement along these collective degrees of freedom the optimization space is reduced tremendously. The refined energy is restricted to the non-bonded ligand-receptor classical (van der Waals and electrostatic) interactions, requiring only modest computational efforts. For a test set of six receptors that show significant structural change when going from 'open' to 'closed' form, our refinement scheme reduces the coordinate root-mean-square values by 0.3–3.2 Å when between one and five non-trivial normal modes are used to optimize the structure after docking. By including the lowest six rigid-body normal modes the method can also correct mispositioning of the ligand relative to the receptor, with only slightly lower efficiency compared to ideal starting positions. Applications to protein-DNA complexes will also be presented.

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Cryoenzymic studies on yeast 3-phophoglycerate kinase

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A way to build an enzyme reaction pathway is to scrutinize the available structural data, paying particular attention to substrate-induced structural changes because these could give rise to measurable kinetics and the existence of particular intermediates. Here, we exploit this approach with the glycolytic enzyme, 3-phophoglycerate kinase (PGK): ADP + 1,3-biphosphoglycerate(bPG) $\leftrightarrow$ ATP + 3-phosphoglycerate(PG). Extensive studies have been carried out on the structure of PGK, but little is known on its reaction pathway. Because of the instability of bPG, the reaction was studied in the reverse direction, i.e. with ATP and PG as substrates. From structural studies, the PG and ATP sites on PGK are too far away from each other to allow a direct phosphoryl transfer. We propose the following scenario. Upon the binding of PG and ATP, there is a two-stage hinge-bending motion by which the substrates are engulfed and the catalytic site formed. Following the phosphoryl transfer, the ternary complex PGK.bPG.ADP isomerizes by a small structural change that favours the release of products in the order ADP first and then bPG. We tested this pathway by a detailed transient kinetic study using chemical sampling techniques in the msec time range. The problems of rapidity of the reaction and unfavourable equilibria were overcome by addition of solvent (30% methanol) and low temperature, i.e. cryoenzymology. The data fit well to the structurally inspired scheme and the kinetic constants describing it were estimated.

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Molecular order and structure of spider dragline silk studied by two-dimensional solid-state NMR

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Spider dragline silk is known for its exceptional elasticity and tensile strength. It is composed of two proteins, spidroin I and II, which contain poly-Alanine blocks and Glycine-rich domains. Some models attribute the elasticity of dragline silk to amorphous Gly regions and toughness, to crystalline Ala-rich parts. The goal of our work is to understand this silk at a molecular level. More specifically, we have investigated the molecular order and structure of dragline silk produced by the orb-weaving spider *Nephila edulis*. To do so, we have used two-dimensional ^{13}C-^{13}C spin diffusion experiments with MAS and ^{13}C-labelled silk. First, from the intra-residue cross peaks observed on the correlation spectra, the chemical shift assignment of the silk sample is presented. The chemical shift values evidence that residues such as Gly and Ala are not confined to unique domains of silk but are found in various environments (β-sheets, helices). Interestingly, the cross peak shape reveals that even the microcrystalline Alanine domains present a certain degree of molecular disorder. Inter-residue correlation peaks are observed at long spin-diffusion times ($\geq$ 500 ms), allowing distance calculation. Finally, the effect of thread stretching on the molecular order of silk is discussed.

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2D crystallization of ferritin molecules by means of target-specific affinity peptides

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We have been proposing a new concept for making inorganic nanostructures named "Bio Nano Process" (BNP), which exploits self-assembly and biomineralization of bio molecules (*THIN SOLID FILMS*, **393** (2001), 12). It will realize the bottom-up construction of the key-components of nano-electronic devices and be a novel manufacturing technique for the semiconductor industry. One application of the BNP is making the floating nanodots gate memory, which needs closely packed nanodot array embedded in the insulator layer. To realize such nanodots array, 2D-crystal of ferritins should be formed on the Si substrates. The modification of the protein surface by peptides with specific affinity for the substrate could govern the protein-protein/protein-substrate interactions and would make it possible to form 2D ferritin crystal directly on the Si substrate. By fusing the ferritin with Carbon Nano horn binding peptides (*Langmuir*, **20** (2004), 8939) and the survey of appropriate ferritin adsorption conditions, we have successfully made 2D array of the ferritin on the Si substrate deposited with carbon thin layer. This 2D crystallization involves the attraction between specific affinity peptide and the hydrophilic carbon surface. This success of direct 2D crystallization of ferritin molecules on the Si substrate is the first step to constructing nano structures by the BNP. This work is partly supported by MEXT, Japan.

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Recent advances in neutron macromolecule crystallography with LADI

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Protons and water molecules play critical roles in the enzymatic mechanism of many enzymes. Direct information on hydrogen positions can be obtained from neutron crystallographic data at modest resolutions of 2.5-2.0 Å. Neutron crystallography is therefore a useful experimental tool to address specific problems where direct visualisation of hydrogen atoms is crucial. However, neutron macromolecule crystallography raises many challenges. Unusually large crystals (> 1 mm^3) are required to compensate the weak flux of available neutron beams. Moreover, the large hydrogen incoherent scattering background significantly reduces the signal to noise.
Recent advances in instrument technologies in parallel with the development of the preparation of 100% D-labelled macromolecules make neutron crystallography an appealing technique to address mechanistic and hydration questions on larger biological system and smaller crystal than previously possible. The use of cryo-neutron crystalllography makes now possible to capture transient species enabling intermediary states in reaction pathways to be analysed.
We will discuss recent examples of neutron protein structures determined with the Laue Diffractomer (LADI) neutron-sensitive image plate detector at the ILL. The upgrade of the LADI, producing a minimum 6-fold gain will be presented.

P-142

A pressure dependent change in protein dynamics at 4kbar

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Molecular dynamics simulations of a crystalline protein, Staphylococcal nuclease, have been performed in the pressure range 1bar to 15kbar and at a temperature of 300K and the protein and solvent dynamics have been analysed in terms of average atomic mean-square displacements, X-ray diffuse scattering, the vibrational density of states and principal component modes. The analysis revealed the existence of two linear regimes with the transition being located at ∼4kbar.

P-143

Identifying specific collective motions in Staphylococcal nuclease by X-ray diffuse scattering

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The internal collective motions dominating the X-ray diffuse scattering by crystals of a protein, Staphylococcal nuclease, are determined using molecular dynamics simulation. The basis scattering consists of a smooth, isotropic high-intensity ring at q=0.28A^{-1} upon which intense three-dimensional structured features are superposed. The smooth ring consists of equal contributions from nearest-neighbour water molecule correlations and internal protein motions, the latter consisting of fluctuations in alpha-helix pitch and beta-interstrand distances. Superposed on the isotropic ring are intense, three-dimensional scattering features that can be described using a small number of slowly-varying principal components. The individual three-dimensional features are assigned to specific collective motions in the protein, some of which explicitly involve active-site deformations.

P-144

Synergy of substrate binding, base flipping and catalytic loop motions in a DNA methyltransferase

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The HhaI methyltransferase (M.HhaI) transfers a methyl group from cofactor AdoMet onto its target cytosine residue in DNA. Crystal structures revealed that M.HhaI flips its target base out of the DNA helix. This transition is accompanied by a massive motion of a loop in the protein which locks the flipped out base in the catalytic site.
We used fluorescence of a unique tryptophan residue to specifically monitor cofactor binding. Equilibrium binding studies revealed a highly improved binding of the cofactor AdoMet and the reaction product AdoHcy in the presence of specific DNA. No such effect was observed with non-specific DNA in the case of AdoMet, but, surprisingly, led to a substantial drop in binding affinity in the case of AdoHcy! To elucidate the role of the catalytic loop in substrate binding we constructed two variants of M.HhaI in which large segments of the catalytic loop were entirely removed. Although the binary interactions with the substrates and base flipping was almost unaffected by the deletions, the synergy of substrate binding in the ternary complexes were completely lost. To 'visualize' the loop motions directly during the reaction cycle we prepared a series of double mutants in which a unique tryptophan residue was placed in selected positions on the mobile catalytic loop. Single turnover stopped flow kinetic studies of the mutants revealed two conformational transitions of the loop which coincide with the formation of the tight ternary complex and the release of products.

Posters
– Protein Reactivity and Dynamics –

P-145

Characterization and structural basis analysis of *Escherichia coli* Dps

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Dps (DNA-binding proteins from starved cells) class of non-specific DNA binding proteins assemble to form a shell-like structure of 12 subunits related by 3 : 2 tetrahedral symmetry and contain an iron oxide core. *E. coli* Dps shows a dual mechanism for DNA protection, one is attributed to the physical association of two macromolecules Dps and DNA, and the other is to chelating ferrous iron to prevent oxidative cleavage of DNA. *E. coli* Dps possesses the positively charged N-terminal tail of amino acid residues and it has been proposed to participate in stabilization of the complex with DNA but little is known about the effect on biomineralization.
In order to investigate the effect of N-terminal residues of *E. coli* Dps on the two DNA-protection mechanisms, we constructed four deletion mutants and decided their functional and structural properties *in vitro*. The constructed mutants are less or lack of their DNA-binding affinities because of the lack of their abilities of self-aggregation and DNA condensation. On the contrary, they keep maintaining the abilities to efficiently oxidize iron (II) and protect DNA against oxidative cleavage.
From the present experimental data, two DNA-protection processes are not parallel, and they have little influence on each other.
This work was partly supported by Japan Science and Technology Agency.

P-146

Unravelling Hot Spots– an improved methodological approach

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Alanine scanning mutagenesis at protein interfaces has allowed the discovery of energetically determinant, compact, centralized regions of residues crucial for protein association, which had been defined as hot spots. Binding free energies have been estimated with reasonable accuracy with empirical methods, such as molecular mechanics/Poisson-Boltzmann surface area (MM-PBSA) [1], and with more rigorous computational approaches like free energy perturbation (FEP) and thermodynamic integration (TI). The main objective of this work is the development of an improved methodological approach, with less computational cost, that predicts accurately differences in binding free energies between the wild-type and alanine mutated complexes ($\Delta\Delta G_{binding}$). The computational method presented here achieved an overall success rate of 80% and a 100% success rate in residues for which alanine mutation causes an increase in the binding free energy higher than 4.0 kcal/mol (hot spots). This method reproduces the quantitative free energy differences obtained from experimental mutagenesis procedures, and therefore can be applied to a wide range of protein-protein complexes being of crucial importance of Structure Based Drug Design.
References:
[1] Huo S, Massova I, Kollman PA. Computational alanine scanning of the 1:1 human growth hormone-receptor complex. (2002) J Comput Chem. Jan 15;23(1):15-27.

P-147

Fluorescence dynamics map reveals the pathway of protein amyloid fibril formation and structure

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Protein aggregation leading to amyloid fibrillation has drawn considerable attention in recent times due to its involvement in a variety of neuro-degenerative diseases. In this work, we report the *first* investigation of residue-specific fluorescence dynamics as a novel approach to characterize the formation of amyloid fibrils. Barstar, a model protein used extensively in folding studies, forms amyloid-type fibrils at low (< 3) pH and temperatures above 50°C.
Seven single cysteine-containing mutant forms of barstar were made, and each was labeled with the thiol-active fluorescent probe, IAEDANS. Additionally, Trp53 was also used as an intrinsic fluorescence probe. Picosecond time-resolved fluorescence was used to monitor rotational dynamics at these eight positions in the low pH A-form, and in amyloid fibrils. Based on this, we contruct a dynamic-amplitude map, which illustrates structural fine points along the polypeptide. This map is further supported by measurements of solvent exposure, using dynamic quenching of by acrylamide. The map points out that the N-terminus is less involved in fibrillation when compared to the C-terminal region. Furthermore, it is shown that the transformation of the A-form into amyloid does not alter significantly the local structure at these positions, and hence, occurs directly.

P-148

Energetics and mechanism of Ca^{2+} displacement by lanthanides in a calcium binding protein

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The displacement of Ca^{2+} by trivalent lanthanide ions Tm^{3+} in a protozoan (*Entamoeba histolytica*) Ca^{2+} binding protein has been studied by NMR and isothermal calorimetry (ITC). The study provides a basis for understanding the behavior of lanthanides when used as a substitute for Ca^{2+}, the pattern of sequential binding, the structural changes involved, the range and magnitude of paramagnetic interaction, and the associated energetics and mechanism. The progressive Ca^{2+} displacement from site III first, followed by displacement from site II, I, and IV, as observed during the NMR titration experiments, is interpreted in the light of ITC data to provide a deeper insight into the intradomain and, for the first time, interdomain cooperativity and information about the statistical phenomenon involved in it. A theoretical model governing Ca^{2+} displacement is provided. Quantification of Ca^{2+} specificity against lanthanide displacement and probability in each microscopic binding pathways involved in such displacement processes has been projected here in great detail.

Posters
– Protein Reactivity and Dynamics –

P-149

Structural basis for protein cleavage and green-to-red conversion of fluorescent protein EosFP

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Genetically encoded fusion constructs derived from fluorescent proteins (FPs) can be designed to report on a multitude of events and signals in cells, tissues and entire organs without interfering with the complex machinery of life. The toolbox of FPs, initially limited to the green fluorescent protein (GFP) from the luminescent jellyfish *Aequoria victoria* and its genetically engineered variants, has been greatly expanded in recent years by the cloning of FPs from Anthozoa animals. EosFP is a novel fluorescent protein from the scleractinian coral *Lobophyllia hemprichii* that switches its fluorescence emission from green (516 nm) to red (581 nm) upon irradiation with ∼400-nm light. This property enables localized tagging of proteins. Here we present the X-ray structures of the green and red forms of wild-type EosFP. They reveal that formation of the red chromophore is associated with cleavage of the peptide backbone, with surprisingly little change elsewhere in the structure, and provide new insights into the mechanism that generates this interesting post-translational polypeptide modification.

P-150

Dynamic Stokes shift and ultrafast hydration of proteins

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Biological macromolecules are very well adapted to their local environment, be it the hydrophobic interior of a lipid bilayer membrane or the aqueous cytoplasm inside the cell. The role of water is fundamental in this respect, and the hydration/solvation characteristics of proteins thus have attracted considerable attention since the first realization of the importance of the hydrophobic effect for protein folding. Water is however a complex liquid in itself, and it has turned out to be quite elusive to obtain a detailed picture of its interactions with biomolecules, and not free of contradictions.
The observed 10-100 fold slow-down of the dynamic Stokes shift (DSS) relaxation when a chromophore is bound to a macromolecule has been interpreted in terms of a layer of "biological water" surrounding the macromolecule, and in slow exchange with bulk [*Proc. Natl. Acad. Sci.* (2002) **99,** 1763-1768]. This contrasts with magnetic relaxation dispersion (MRD) data, which show a modest 2-3 fold retardation of the solvent dynamics near the macromolecule [*Proc. Natl. Acad. Sci.* (2003) **100,** 12135-12140]. To clarify this issue we have performed MD simulations on proteins in explicit water and find that the slow component of the DSS relaxation is mainly due to chromophore and protein motions, whereas water relaxation around the protein is in general quite fast, consistent with the MRD data and other MD simulations of water dynamics.

P-151

Unstable structures working: Evidences

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It is reasonable to think that the less stable structures in solution may contribute the less to the biological functions due to its short life time (*i.e.*, another expression of *instability*). Moreover, less stable structures are more difficult to analyze as if swift swallows were hard to catch. Thus, it is natural that scientists have paid less attention to such unstable (say, less than milli-or-micro second life time) structures except studies on protein folding as intermediate structures. However, the situation is changing owing to the development of science and technology and, thus, the matters which were thought valueless or impossible to attack formerly have been becoming challenge-worthy. Technologies such as femto-second laser technology and reliable MD simulations are currently most useful for this purpose. On the other hand, the conformational diversities of biopolymers (DNA/RNA, peptide, and protein) can be high-lighted from some reasons. In this paper, we discuss the solution structure dynamics of oligonucleotides, peptides, and single-stranded DNAs based on the observations obtained by hydrodynamic experiments, molecular dynamics, and enzyme-monitored solution structures, referring to the topics such as SSCP (single-strand conformation polymorphism) and small RNAs (*i.e.*, novel knowledge conveyed by genomics).

P-152

Structure in protein aggregation disease: Entropic contributions to stability of conformations

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Huntington's Disease is the most common inherited neurodegenerative disorder. It is caused by a mutation in the polyglutamine tract of exon-1 in huntingtin. Carriers with 40 or more glutamines (Q) develop the disease within a normal lifespan. Certain neurons form ß-amyloid fibrils and die. Soluble oligomers precede fibrils, and evidence shows they are the actual toxic species. Kinetic studies of polyQ show that the critical nucleus for aggregation is a monomer, with circular dichroism spectra characteristic of ß-structures. Those facts prompted the simulation of a polyQ monomer of 40 residues with classical molecular dynamics to explore its possible conformations. Several Charmm force fields were used, some containing a quantum mechanical correction for the protein backbone energy. Both enthalpic and entropic contributions to the free energy are calculated to gauge the stability of a dozen putative structures. The most promising one is a triangular ß-helix with parallel contacts derived by threading using coordinates for 1QRE, a thermophilic carbonic anhydrase.

Posters
– Protein Reactivity and Dynamics –

P-153

Reactive molecular dynamics simulations of MbNO

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Hemoglobin and myoglobin are involved in the biological transport and storage of oxygen. Due to their physiological importance, the interaction of these proteins with their ligands (O_2, CO, NO) has been extensively studied both experimentally and theoretically. A phenomenon of special interest is the reversible binding of the diatomic molecules to the heme unit. Depending upon the ligand, the time scales for the rebinding differ by orders of magnitude. In the case of NO, the time scales involved are in the ps range and thus amenable to detailed molecular dynamics simulations.
Using a recently calculated interaction potential for the bound state of MbNO state and a potential for the unbound Mb...NO state, the actual rebinding process is studied. To connect the two asymptotes(bound/unbound) a classical switching mechanism that connects the two potential energy manifolds is used. The algorithm conserves energy and provides lower estimates for the reaction rates.
Several hundred trajectories starting from the dissociated Mb...NO state are followed. Their rebinding times are determined and statistically analysed. In agreement with experiment, nonexponential rebinding dynamics is found. The simulations suggest that, in addition to relaxation and possible multiple binding sites, the existence of a metastable bound MbON conformation contributes to the nonexponential relaxation.

P-154

Experimental confirming of the soliton model of the bio-energy Transport

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Spectra of infrared absorption of collagen and bovine serum albumin with α-helix structure are collected by FT-IR spectrometers at 300K in the region of 400-4000 cm^{-1}. This result in the spectra of infrared absorption of the proteins show that (1) a new 1650 cm^{-1} band, corresponding to solitons in the soliton theory, close to the amide-I band 1666 cm^{-1}, corresponding to exciton, for vibration of amide-I are found; (2) the properties of exponential decrease of intensity for 1650 cm^{-1} anomalous band, $\exp[-(0.437+8.987\times10^{-6}T^2)]$, and of linear increase of intensity for 1666 cm^{-1} band with increasing of temperature in collagen are obtained, which resemble with the results by the soliton theory; (3) the bands of 1680.31, 1666, 1650, 1624, 3209, 3225, 3244, 3262, 3278, 3296, 3316, 3333 and 3355 cm^{-1} occurred in the absorption spectra in the regions of 1000-4000 cm^{-1} coincide with theoretical values of 1667, 1662, 1653, 1627, 3204.7, 3218.2, 3242.5, 3261.8, 3278.9, 3298.9, 3313.4, 3333.9 and 3358.6 cm^{-1} obtained by Pang's model with quasi-coherent two-quantum state and containing various interactions for bio-energy transport in á-helix proteins with three channels. The experimental results confirm that the soliton transporting bio-energy in the proteins and Pang's theory is correct.

P-155

Molecular dynamics investigation of effects of calcium ion on structure and autolysis of trypsins

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The calcium ion was proposed to be involved in protein structure stabilization against thermal and proteolytic degradation, such as autolysis phenomena, in trypsin-like serine proteases. However, molecular and structural details related to the role played by the metal ion are still largely unknown.
Molecular dynamics simulation provides a powerful tool to understand the dynamics of a protein in atomic details which can lead to significant insights into the atomic motions and the machinery underlying the protein function. Several molecular dynamics simulations of 6 ns have been used to investigate the dynamics behaviour of mammalian trypsins in calcium-bound and calcium-free forms, with the aim of evaluating the role of calcium ion on trypsin three-dimensional structure and autoproteolysis propensity.
It turned out that the calcium-free trypsins are characterized by a more disordered three-dimensional structure. Moreover, the removal of calcium ion not only increases the flexibility of regions around its binding site but, also leads to channeling of the fluctuations to remote sites. This observation agrees with other general mechanisms by which the signal induced by metal binding is transmitted to distant regions in the three-dimensional structure. In particular, some primary autolysis sites are strongly influenced by calcium binding and their flexibility, rather than their solvent accessibility degree, is proposed to be influenced by calcium ion removal.

P-156

Molecular dynamics simulations to study enzyme cold-adaptation: a family-centred point of view

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In recent years, there has been increased interest in the origin of enzyme adaptation to low temperature both for understanding the protein folding and designing of biocatalysts with enhanced activity and thermostability.
The number of reports on enzymes from cold adapted organisms has increased significantly over the past years, and reveals that adaptative strategies varies among enzymes, which use different small selections of structural features for gaining increased molecular flexibility that in turn lead to increased catalytic efficiency and reduced stability.
Molecular flexibility is a parameter difficult to estimate by experimental methods, whereas molecular dynamics simulation of protein systems provides a suitable tool to evaluate flexibility and molecular properties of proteins and correlate them to protein structural and functional aspects. In the present contribution we report results obtained from several long molecular dynamics simulations of representative structures for mesophilic and psychrophilic enzymes at different temperatures, to explore the molecular basis of cold adaptation inside a specific enzymatic class. The molecular dynamics trajectories were compared and analyzed in terms of secondary structure contents, molecular flexibility, intramolecular interactions and protein-solvent interactions, unravelling putative structural and molecular determinants of thermolability and activity at low temperature for psychrophilic enzymes.

Posters
– Protein Reactivity and Dynamics –

P-157

Protein dynamics in very different time windows

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Many proteins resemble tiny engines on the scale of nanometers. For their function internal motions are necessary, occurring on very different time scales. At un-physiologically low temperatures, the molecules are in a "stand by" state. Above a characteristic temperature, T_c, slow protein specific motions become measurable allowing the molecular engine to fulfill its function. The fastest motions (1 fs to 0.6 ps) which are present in the entire temperature range from cryogenic to room temperatures are intramolecular vibrations which can be analyzed by “Phonon Assisted Mössbauer Effect” using synchrotron radiation. Combining the results of neutron structure analysis and incoherent neutron scattering allows to separate 3 types of hydrogen mean square displacements above T_c: backbone like (slower than about 100ps), methyl like (partly slower partly faster than about 100ps) and lysine like (faster than about 100ps). Mössbauer spectroscopy on the heme iron reveals diffusion like motions of molecular segments in a time range between 6 and 1ns. Such motions are necessary for conformational changes. This is shown for the structural relaxation of myoglobin and haemoglobin after photolysis of a ligand or after creating a metastable intermediate by irradiations with X-rays. For an introduction compare:
F.G. Parak, Rep. Progr. Phys. 66:103-129, (2003) and
F.G. Parak, Current Opinion in Structural Biology 13:552-557 (2003)

P-158

Affinity-directed reconstitution of membrane proteins into polymer supported lipid bilayers

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Reconstitution of membrane proteins into solid-supported membranes is a key issue for biophysical analysis of their function *in vitro*. While several successful attempts have been described, no simple and generic methodology is currently available. Here, we describe a generic method for membrane protein reconstitution into solid-supported membranes, which uses reversible affinity-capturing of the membrane protein from detergent solution followed by lipid bilayer assembling from mixed micelles. A surface architecture on glass supports was developed, presenting high-affinity multivalent chelator groups (MCH) and hydrophobic anchoring groups on a ultrathin oligo(ethylene glycol) brush in an adjustable format. Thus, membrane proteins can be incorporated into membranes in a defined concentration and orientation. Protein immobilization and reconstitution into the lipid bilayer was monitored by simultaneous detection by total internal reflection fluorescence (TIRF) and reflectometric interference (RIf). Mass-sensitive detection by RIf supported formation of a lipid bilayer by this reconstitution method, but the fluidity of these membranes as studied by FRAP was low. Transmembrane receptors maintained their ligand binding affinity during this procedure, suggesting oriented incorporation into the lipid bilayer. Detachment of the reconstituted protein from the MCH headgroup by removing the chelated metal ions was monitored by de-quenching of a fluorophore attached close to the histidine-tag.

P-159

Time-resolved resonance Raman and FTIR studies of nitric oxide reductase and heme-copper oxidases

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Time-resolved resonance Raman (TR^3) and time-resolved step-scan (TRS^2) FTIR spectroscopies have been used to probe the structural dynamics at the heme b_3 proximal and distal sites subsequent to carbon monoxide photolysis from fully reduced CO-bound nitric oxide reductase (NOR) and heme-copper oxidases (CcO). The TR^3 spectra of NOR exhibit structural differences relative to the equilibrium geometry of heme b_3. The most significant of these is a shift of 8 cm^{-1} to higher frequency of the 207 cm^{-1} mode that originates from ν(Fe-His), and a shift of 7 cm^{-1} to lower frequency of the ν_4 mode. We suggest that relaxation along the tilt angle of the proximal histidine with respect to the heme plane and the out-of-plane displacement of the Fe (q) are coupled, and ligand binding and dissociation are accompanied by significant changes in the angular orientation of the His ligand. The results are compared to those obtained for heme-copper oxidases. The TR^3 and TRS^2 FTIR data demonstrate significant alterations in the nature of the heme-protein dynamics between NOR and CcO resulting from specific structural differences in their respective hemepockets.

P-160

Detection of a docking site in the oxygen-sensing signal transducer protein HemAT

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The structure determination of ligand binding intermediates in proteins is the key step toward our understanding of ligand binding and discrimination. We have used FTIR and time-resolved step-scan FTIR spectroscopy on CO–bound and –photolyzed HemAT, a newly discovered signal transducer heme protein that detects O_2, to gain insight into the structure of ligand binding intermediates at ambient temperature. We show that upon photodissociation, the photolyzed CO becomes trapped within a docking site located in the vicinity of the O_2 regulating binding distal residue Y70. The 2065 cm^{-1} mode of the “docked” CO indicates that the ligand exhibits strong H-bonding interactions that substantially weaken the C-O bond. We suggest that Thr95, Leu92 and Val89 provide the H-bonding network on the “docked CO. The results of the CO-bound Y70F mutant indicate that HemAT has a pre-existing docking cavity that is modestly perturbed by the photodissociated ligand.

Posters
– Protein Reactivity and Dynamics –

P-161

Nitrophorin 4, protein dynamics in a beta-sheet protein

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Nitrophorins are the heme proteins which have an almost pure beta-sheet structure. They use heme Fe(III) for reversible binding and transport of NO. We report Mössbauer and optical studies of nitrophorin 4 (NP4). In NP4 the heme is attached through the proximal histidine HIS59 and the heme iron can bind a 6^{th} ligand such as NO, NH_3 or others. Mössbauer studies of NP4 show a linear increase with temperature of the dynamic mean square displacement, $<x^2>$, of the iron below 200K and a dramatic increase above 220K, a characteristic temperature that is 30K higher than in alpha-helical myoglobin. NP4 in water-glycerol solution shows optical spectra of high-spin Fe(III) with the Soret band at 403 and 405nm at 300 and 20K, respectively. The laser photoinduced reduction of NP4 with tris-2,2'-bipyridine ruthenium(II) at 20K produces an intermediate state with the Soret band at 419nm and Q bands at 555 and 521nm indicating a low-spin Fe(II) and the presence of a water as a 6^{th} ligand of the iron. Above 160K the intermediate relaxes to the initial NP4 Fe(III) state in contrast to the low-spin Fe(II) intermediate of myoglobin that relaxes to high-spin Fe(II) deoxymyoglobin[1].

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P-162

Real-time detection of time-resolved dynamics changes of membrane receptors during function

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Advanced multidimensional time-correlated single photon counting (TCSPC) in combination with time-resolved fluorescence depolarization is a valuable tool to study the dynamics of membrane protein surface segments on the pico- to nanosecond timescale.
The elucidation of the nature of conformational changes provides a basis for the understanding of the molecular mechanism underlying the activation of G-protein coupled receptors, as shown for rhodopsin. We were able to identify two mechanisms of loop conformational changes in the functionally intact proteins bacteriorhodopsin (bR) and bovine rhodopsin: we found a surface potential change-based switch between two conformational states of the EF-loop of bR and a striking pH-dependent conformational change of the fourth loop on the cytoplasmic surface of rhodopsin (1).
The functional significance of the detected mechanism of conformational changes was determined by real-time monitoring of the time-resolved anisotropy decay of the fluorescent dye during the time course of the photoreaction of rhodopsins. This is the first experiment, which assesses directly be means of "pump-probe" experiments the diffusional dynamics of the intracellular segments during rhodopsin function. We are able to correlate key events in receptor activation with the time course of loop dynamics changes and to provide new insights into dynamics-function relations.

(1) U. Alexiev, I. Rimke and T. Pöhlmann (2003). *J. Mol. Biol.* **328**, 705-719.

P-163

Fluorescence recovery after photobleaching : a new method to analyse interactions between proteins

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Pseudomonas Aeruginosa is a Gram-negative bacteria. It develops a multidrug resistance to antibiotics. This resistance owes much to efflux pumps which take toxins out of the cell. They are built on three proteins, one of the major association is the MexA/B/OprM pump. It consists of the inner membrane proton-antibiotics antiporter protein MexB, the outer membrane associated protein OprM and the membrane fusion protein MexA which is assumed to connect the two others proteins. Our aim is to understand the way the proteins interact to find their functions and to test pump inhibitors. First, we decided to focus on the periplasmic protein MexA. We study its interaction with the outer membrane protein OprM to know whether MexA is involved in the recruitment of this protein.
To analyse binding reactions we select the study of lateral mobility by photobleaching methods. What are the advantages of such a process compared to well-known experiments such as Biacore?
First, proteins are incorporated in model bilayers so it represents a biomimetic membrane. Furthermore this system allows us to modify the water size between bilayers. So we can test the size and the orientation of the interaction. Thanks to this experiment, we manage to measure unbiding times between two components of the pump MexA and OprM in order to access to interaction energy on the two associated proteins.

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One and two iron center superoxide reductases from *A. fulgidus*: mechanistic studies

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Superoxide reductases (SORs) are mononuclear iron proteins present in many prokaryotic anaerobes and microaerophilic bacteria that scavenge the superoxide radical by reducing it to hydrogen peroxide. These enzymes show a very high reactivity towards superoxide with a nearly diffusion-limited second order rate constant of 10^9 $M^{-1}s^{-1}$. Nevertheless, the overall molecular mechanism of the catalyzed reaction is still a matter of debate, due to the lack of evidences for the identification of the intermediary species formed. We studied the reactivity of SORs from the hypertermophilic archaeon *Archaeoglobus fulgidus* using the pulsed radiolysis technique. Intermediate species formed during the reaction were detected and characterized by visible spectroscopy. Further insight about the identification of those species was obtained by using site-directed mutants without the iron sixth ligand. Based on the assignment of the transient species to different protein states, a new proposal for the mechanism of superoxide reduction by SOR is made.

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– Protein Reactivity and Dynamics –

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The characteristics of polyphenol oxidase in Crocus sativus L. corm

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Polyphenol oxidases (PPOs) catalyze the oxidation of phenols into o-quinones with concomitant oxygen reduction, producing the dark colour associated with fruits, hair, etc. PPOs may also play a role in resistance to diseases. An extract obtained from corms of *Crocus sativus* L., the plant that produces saffron, showed high PPO activity with some substrates. The rate of substrate oxidation was pyrogallol > p-cresol > L-DOPA > L-tyrosine. Apparent Km and Vmax/mg prot were, respectively, 0.26 mM and 12.4 mM/min for pyrogallol, 83 mM and 0.06 mM/min for p-cresol, 4.3 mM and 0.02 mM/min for L-DOPA. Kojic acid inhibited the activity with IC50 of 0.6 mM with pyrogallol, 0.8 mM with p-cresol and 1 mM with L-dopa. The type of inhibition was mixed with pyrogallol, uncompetitive with p-cresol and noncompetitive with L-dopa. Because of a poor L-tyrosine utilization, kinetics parameters could not be determined accurately for that substrate, including the kojic acid IC50 and type of inhibition. Strong substrate inhibition was observed with p-cresol and pyrogallol, but not with L-dopa. Michaelis-Menten plot was S-shaped for p-cresol, suggesting a cooperative binding process with this substrate. SDS increased 3-5 times the rate of p-cresol oxidation by a still unknown mechanism. However, even in the presence of SDS, drastic substrate inhibiton was observed.

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Nanofibrillar structure and molecular mobility in spider dragline silk

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Spider dragline silk is a semicrystalline biopolymer made out of an aminoacid sequence with dominating alanine and glycine molecules. It has outstanding mechanical properties despite being spun at nearly ambient temperature and pressure from a watery solution. Recent x-ray (SAXS/WAXS) experiments have suggested the presence of nanofibrillar units composed of crystalline and short-range order domains embedded in a random polymer chain matrix. The nature and mobility of water in dragline silks is largely unknown although the mechanical properties of silk are strongly influenced by the water content and water-induced mobility at a molecular level. We have recently established for highly aligned spider dragline silk bundles that neutron diffraction (SANS/WANS) techniques provide unique new information on the nanofibrillar structure and its accessibility to water. Thus water is apparently reversibly absorbed by the random polymer chains and the short-range order domains whereas the crystalline domains are not accessible to water molecules. We have also investigated water mobility and water-enhanced amorphous polymer chain mobility by wavevector-resolved neutron spectroscopy as a function of temperature and humidity, thus complementing existing experiments on molecular dynamics (NMR) towards ns and ps time scales.

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Conformational studies of fragment 318-327 of gp120 of HIV-1 using NMR and molecular dynamics

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The V3 loop of the outer envelope (gp120) of HIV-1 has been mapped as the Principal Neutralizing Determinant of the virus. This acts as an immunogen, able to induce a cytotoxic response that can kill infected cells. It represents an important target for vaccine development, but being a hypervariable region there is a glitch in the development of a vaccine. It contains a stretch of a highly conserved GPGR sequence at its tip. Although the biological significance of this conserved sequence is unknown, the adoption of a conserved secondary structure, type II β-turn has been proposed to be responsible for its action.
To probe the effect of the conserved sequence upon conformational adaptability of the V3 loop, we have studied a truncated fragment containing the GPGR sequence [fragment (318-327); A**GPGA**AFVTI] by NMR and MD simulations. Experiments have been carried to get temp. coeff., $^3J_{NH\alpha}$, CSI, dihedral and distance restrains. These were incorporated in a restrained MD simulations to generate the structure. Results indicate that the fragment (318-327) of gp120 adopts a random coil structure. This shows that the suggested β-turn may not be important for its biological activity.

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Role of dynamics in the influence of allosteric effectors on the oxygen binding of Human Hemoglobin

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The conformational dynamics of HbA in the oxy and deoxy state, and bound to the allosteric effectors Cl-, IHP, DPG, BZF/RSR13 where studied by computational and/or experimental methods. The experiments were based on the fluorescence of Trps and Zn-PP in alpha and beta hybrids of oxyHb, synthesized to model the T-state. Line narrowed fluorescence spectra at 10 K measured as a function of hydrostatic pressure up to 15 kbars were used to determine the internal isothermal compressibility of the protein matrix, and fluorescence at room temperature to study the dimer/tetramer stability under pressure under various conditions. The computational simulations were performed on the tetramers under explicit solvation and periodic boundary conditions. Molecular dynamics trajectories (CHARMM) were acquired on equilibrated structures for 1 ns using CHARMM and NAMD. The subunit interfaces were analysed and showed significant differences with respect to X-ray data concerning the interactions stabilizing the tetramer. The characterization based on modeling agreed well with experimental results that showed increased stability with the effectors bound. The overall molecular dynamics was also influenced by the effectors, the RMSD was increased. The compressibility parameter showed this tendency only in the case of Cl- as effector.

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Functional analysis of diguanylate cyclase PleD by molecular dynamics simulations

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The signaling compound cyclic di-guanosine-monophosphate (c-diGMP) plays an important role in modulating bacterial growth on surfaces which leads to infectious diseases and the life-threatening colonization of medical devices. Thus guanylate cyclases are potential targets to combat biofilm-related infections. A first step to understand suppression of biofilm-formation has been the crystal structure of PleD from *C. crescentus* co-crystallized with its product c-diGMP located an allosteric site at the stem-DGC interface.
Molecular dynamics is an ideal tool to provide insight into the mechanism of feedback inhibition. The character of low frequency modes is correlated with significant conformational transitions important for function. The change of protein dynamics under the influence of the ligand identifies secondary structure elements related to protein function and possibly yields information on the dynamical correlation between the inhibition-and the active-site. Correlated motions were found within and between the three domains of PleD. They identify in detail residues essential to signal transduction either within the catalytically active domain or from the distant domain where activation via phosphorylation occurs. Results on the Mutant R390A that has a much lower affinity for c-diGMP binding will also be presented.
Finally, we present results on the functional modes and report on their influence on the suggested dimerization interface which determines a way to predict binding affinities for nucleotide derivatives.

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Control of protein function by autolytic cleavage of disulfide bonds

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The presence of disulfide bonds in proteins has long been understood to provide a structural role, stabilizing the protein tertiary structure. It has recently been realized that some disulfide bonds have evolved to control how proteins work by breaking or forming in a dynamic and precise way. While some proteins may depend on external oxidants/reductants to drive the disulfide bond formation/cleavage, it is to be expected that these effectors may not always be available. In particular, in the extracellular milieu, such effectors will be limited. Data mining of disulfide-containing proteins coupled with quantum chemical calculations suggest a YE(D)YK(R) motif that can facilitate disulfide bond cleavage, directed by small structural changes probably induced by protein binding. These changes reorient residues of the YE(D)YK(R) motif near the disulfide bond and have two effects: polarization of the disulfide bond by the base residue which makes the bond more susceptible to hydrolytic attack, and creating a hydrogen-bonded chain, through the two tyrosines and the acid, that allows for rapid protonation of the thiols so bond cleavage can occur. The protein Semaphorin 3A is used an example of the details of this mechanism.

P-171

Pressure effects on ligand exchange across the protein-solvent interface and binding kinetics in myoglobin

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In heme proteins, pressure in the 200 MPa range causes subtle conformational changes, and it affects the binding kinetics of CO or O_2 to the central iron atom. Flash photolysis experiments performed on myoglobin over wide ranges of time and temperature have shown that ligand binding involves multiple intermediate states. High pressure studies have focussed on either the fast or slow process over a limited time window near room temperature or the geminate process at low temperature.
We present kinetic absorption measurements over eight decades in time of CO and O_2 binding to (horse) myoglobin at variable pressure (0.1 - 190 MPa) and temperature (180 - 300 K) in aqueous and 75 % glycerol/buffer solutions. The data demonstrate that pressure significantly affects the amplitudes (not just the rates) of the component processes. For instance, the number of unbound ligands at 10^{-3} s in the 220 K kinetics is roughly a factor of 10 smaller at 190 MPa than at 0.1 MPa. The amplitude of the geminate process increases with pressure corresponding to a smaller escape fraction of ligands into the solvent and a smaller inner barrier. Specific heat spectroscopy data show that high pressure slows down structural relaxation of the solvent. The significant decrease in the exchange of ligands across the protein-solvent interface suggests that the ligand pathways are controlled by internal cavities in the protein and by the dynamics of the solvent and the hydration shell.

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Low-frequency Raman and Brillouin spectroscopy of lysozyme in solution

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Low-frequency vibrational modes and their dynamic coupling to the solvent are relevant for functional motions of active proteins. A sensitive probe for their dyanmics with high frequency resolution is provided by Raman and Brillouin spectroscopy. We report on polarization resolved measurements of the light scattering spectrum of lysozyme in aqueous solution over the frequency range from 1 GHz to 20 THz and temperatures from 275 to 300 K.
The dynamics extend over more than 3 decades and show temperature independent peaks at 1.5 THz for water and at 2 THz for the protein solution. The alpha-relaxation peaks are observed between 40 and 200 GHz, and these move to lower frequencies with decreasing temperature due to slowing down of structural relaxation. At frequencies below 30 GHz the protein solution shows an excess of quasielastic scattering as measured on both the Stokes and the Anti-Stokes side of the Rayleigh line. The excess intensity is discussed with respect to protein rotational motions and relaxations of the bound water. The apparent compressibility of the protein increases the frequency of the Brillouin line.

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Comparison of the interaction of different cationic peptides with negatively charged membranes

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A sequence of several positively charged amino acids is a common binding motive in membrane bound proteins. We studied the interaction of the positively charged peptides poly(L-lysine) (PLL) and poly(L-arginine) (PLA) with negatively charged DPPG membranes using isothermal titration calorimetry (ITC). We measured directly the binding constant K and the binding enthalpy Δ_RH and calculated Δ_RG, Δ_RS, and $\Delta_R C_p$. This allows us to draw conclusions about the driving forces of binding and the redistribution of counter ions and hydration water.
Differential-Scanning-Calorimetry (DSC) experiments revealed a general increase of the main-transition-temperature (T_m) of DPPG membranes upon binding of PLL, which is not the case for PLA.
From FT-IR spectroscopic experiments we concluded that the binding of PLL/PLA induces a higher order in the hydrophobic part of the membrane, which is probably caused by a decrease of the lipid headgroup area. Furthermore we determined the secondary structure of bound PLL/PLA. Finally we will present clear indications for domain formation in mixed gel state membranes (DPPC/DPPG) upon binding of the peptides.

P-174

Secondary structure and neurotrophic effect of a 35kDa specific protein in spinal sensory ganglia

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A comparison of the proteins expressed between the rat spinal sensory ganglia and motor neurons was made by two dimensional electrophoresis and the result showed that several proteins whose Molecular weight was about 35kDa were present in the spinal sensory ganglia but not in spinal motor neurons. We isolated and purified one of the proteins whose isoelectric point was 5.52 by DEAE-Sephacel ion exchange chromatography and High Performance Liquid Chromatography. Circular dichroism of the purified protein showed in its secondary structure there were 20.8% α-helix, 54.8% β-sheet, 7.3% turn and 17.1% random. Biological experiments found that the protein had neurotrophic effects on the dorsal root ganglia (DRGs) of E-8 chick embryo cultured *in vitro*.The Biological function of this protein is in progress.

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Storage and conversion of energy by proteins

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Enzymes are molecular machines that convert chemical energy into work. It has been suggested [1] that the occurrence of vibrational excited states can be an efficient way of storing the energy in the protein and of transferring it from site to site. The hypothesis that the excited state is a vibrational mode of the peptide group known as Amide I was put forward by Davydov [2]. It has been shown that those excitations can indeed be transferred from site to site, even at biological temperatures, in tens of picoseconds [3]. On the other hand, lifetimes of tens of picoseconds were measured in real proteins and systems alike [4, 5], suggesting that Amide I excitations last long enough in the protein. Davydov's model provides, thus, a possible description for the storage and transport of energy in the protein. However, as a theory for the energetics of proteins, this description is incomplete because the model conserves the number of Amide I excitations. Within this model, once an excitation is created in the protein, it remains and its energy cannot be released for work. Here we are concerned with a non-conservative generalization of Davydov's model.
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P-176

Nucleotide binding to the first ATPase subunit of MRP1 probed by chemical shift mapping

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Multidrug-resistance-associated protein 1 (MRP1/ABCC1) is a member of the human ABC transporter superfamily that confers cell resistance to chemotherapeutic agents. ATP-binding cassette (ABC) transporters couple ATP hydrolysis to the transport of endogenous and exogenous molecules across biological membranes. In contrast to prokaryotic ABC transporters, the ATPase activity of both isolated nucleotide-binding domains (NBD) of MRP1 was found to be extremely low. However fluorescence as well as NMR experiments show that MRP1-NBD1 binds ATP. Having built a homology model, we wanted to get a more detailed insight into nucleotide binding by using NMR chemical shift mapping. The sequential assignment of the backbone resonances of NBD1 is a prerequisite to the interpretation of the ^{15}N-HSQC NMR spectra. It was obtained by applying the usual triple resonance experiments on the ^{13}C-^{15}N-^{2}H-labeled protein domain and assisted by selective in-vitro labeling of several amino acids. This allowed us to identify the residues involved in nucleotide binding (ADP or ATP, in the presence or in the absence of magnesium). They form a binding pocket, which corresponds to the classical ATP-binding site in ABC-transporters. The extent of chemical shift changes also indicates that small conformational changes outside the binding pocket accompany nucleotide binding.

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The carboxylate-shift in FTase - Analysing an intriguing mechanistic feature in a puzzling enzyme

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Farnesyltransferase (FTase) is a zinc enzyme that catalyses the addition of isoprenoid farnesyl, from farnesyldiphosphate (FPP), to a cysteine residue of a protein substrate containing a carboxyl terminal CAAX motif, where C is the cysteine that is farnesylated. During the last decade, FTase has established itself as a very promising target in anticancer research, with more than 100 patents describing FTase inhibitors published since 2000 [1]. Studies in other areas, namely in the fight against parasites and virus, have also led to very promising results. However, in spite of the enormous interest that has been devoted to its study, the mechanism of this enzyme remains the subject of several crucial doubts [1,2].
In the center of the FTase activity lies a covalently coordinated Zn (II) cation, which is known to suffer a carboxylate-shift rearrangement with Asp297 [3,4], both with ligand entrance (bimonodentate) and with product exit (mono-bidentate). This study sheds a new light in this interesting phenomenon, by analysing the energetic advantages that this feature represents in the overall context of the FTase activity, using high-level theoretical calculations.
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Determination of 3 sets of parameters for the molecular modelling of the farnesyltransferase enzyme

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Protein farnesyltransferase (FTase) has established itself as a very promising target in anticancer research, with more than 100 patents describing FTIs published since 2000 [1]. However, several crucial doubts in its catalytic and inhibition mechanisms still remain [2]. This work reports the determination and validation of three sets of molecular mechanics parameters for FTase, committed to the AMBER force field, specifically designed for the three different zinc coordination environments formed during catalysis, which represent a total of four possible targets for the development of FTase inhibitors – FTase resting state, binary complex (FTase-FPP), ternary complex (FTase-FPP-Peptide), and product complex (FTase-Product) – thereby covering the complete mechanistic pathway of this mysterious enzyme. DFT (B3LYP) calculations, crystallographic data [3], and EXAFS [4] results were used in the parameterization, together with new important facts obtained from recent mechanistic studies [5,6].
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Fret studies of structural properties of heparin-binding peptides

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FRET (Förster Resonance Energy Transfer) was applied to study structural properties of heparin-binding sequences (Cardin Motif peptides). Internally quenched fluorogenic peptides were synthesized containing the fluorescent donor *o*-aminobenzoic acid (*o*-Abz) and the acceptor dinitrophenyl ethylenediamine (Eddnp) group. Using the CONTIN computational package distance distributions were recovered from time-resolved fluorescence data, associated to end-to-end distances for several Cardin Motif peptides. The so called RK4, RKK4 and RKK3 peptides have random structure in aqueous medium, with relatively extended conformation. In the presence of heparin, the interaction with the glycosaminoglycan considerably decreased the end-to-end distances, in agreement with circular dichroism data suggesting alpha-helix formation. Distance distributions compatible with compact conformations were also observed in the solvent trifluoroethanol (TFE) and in aqueous suspensions of SDS micelles. Although the peptide acquire shorter end-to-end distances due to interaction with solvent like TFE and with heparin or SDS, the values obtained are smaller than expected for the peptides in alpha helix conformation, indicating the occurrence of bend in the structure, leading to additional decrease in the distances.

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Free energy landscape of protein-protein association resulting from brownian dynamics simulations

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We carefully analyzed the trajectories from Brownian Dynamics (BD) simulations in order to study the details of protein-protein encounter for barnase : barstar, cytochrome *c* : cytochrome *c* oxidase, and adrenodoxin : adrenodoxin reductase. The individual positional and orientational coordinates of the proteins during all trajectories are stored in occupancy maps. By interpreting the occupancy maps as probability distributions and by defining a local entropy function we are able to compute the 6-dimensional entropy landscape for the encounter of the two proteins. Together with the configuration dependent electrostatic and desolvation energies, the landscape of the association free energy is obtained as the sum of these terms. In the free energy profile along the reaction path, which is defined as the path along the minima in the free energy landscape a characteristic minimum at small distances shows up, suggesting this to be used as the definition of the encounter state.
From the occupancy maps and the computed free energy landscape we are able to deduce the pathways of association and the regions of the encounter complex. This analysis therefore enables the clarification of the effects of mutants or of varying ionic strength on the association behavior of the proteins.

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Myoglobin dynamics studied with incoherent neutron scattering

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The function of proteins not only depends on their three dimensional structure but also on their dynamics. Sperm whale myoglobin is used as a representative of globular proteins. A Neutron crystal structure analysis of myoglobin at 1.5 Å resolution was utilized to study the positions and mean square displacements, $\langle x^2 \rangle$, of the hydrogen atoms. It is shown that the $\langle x^2 \rangle$ of the hydrogen atoms can be divided into 3 classes. The comparison of crystallographically obtained $\langle x^2 \rangle$ with dynamic displacements measured by incoherent neutron scattering shows that the side chain hydrogen atoms contribute to a large extent to the dynamic displacements on a time scale faster than 100 ps. To investigate the dynamical behaviour of the active center, the so-called heme group, measurements on fully deuterated (^{2}H) myoglobin with protonated (^{1}H) heme group are in preparation. In this experiment incoherent neutron scattering probes the movement of the heme group on a time scale faster than 13ps. The measured mean square displacements of the heme group will be compared with results from Mössbauer spectroscopy of fully protonated myoglobin.

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Spectrophotometry, circular dichroism and kinetics of the effect of Ni2+ on horseradish peroxidase

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Many heavy metal ions are essential for living systems at trace levels, and toxic at elevated levels. In this work, horseradish peroxidase C (HRPC), a well characterized detoxifying enzyme, and nickel, a highly toxic heavy metal, were used as a model system to study alterations in the enzyme activity and conformation induced by a metal ion. Incubation of HRPC with various Ni^{2+} concentrations for 5 min led to changes in the enzyme absorption spectrum, including variations in the intensities of the Soret, beta and charge transfer (CT1) bands absorption, shift in the Soret, beta and CT1 bands maxima and absorption increase at 275 nm. Changes in the alpha-helical content, as determined by circular dichroism spectroscopy, were also found. In the presence of H_2O_2 and o-dianisidine as substrates, and sufficient amount of enzyme, 1-10 mM nickel enhanced the enzymatic activity, while higher Ni^{2+} concentrations (20-50 mM) inhibited it. The enzyme was inactive after 5 min incubation in 50 mM Ni^{2+}. Prolonged incubation (15 and 30 min) induced inactivation at lower Ni^{2+} concentrations. Hill plots suggested the existence of four potential attachment sites per HRPC molecule, which were sequentially occupied in a dose- and time-dependent manner by Ni^{2+}. Based on our results, a scheme is proposed for HRPC conformational changes in different Ni^{2+} concentrations.

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Adaptation to extreme environments via macromolecular dynamics

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The forces that maintain biological molecular structure and govern atomic motions in macromolecules are “weak” forces because their associated energies are similar to thermal energy at usual temperatures. Fast atomic thermal motions on the picosecond to nanosecond timescale allow macromolecules to achieve the stability and motions, and, therefore, the necessary rigidity and flexibility to perform their biological functions. Neutron spectroscopy is particularly adapted to the study of these motions, because neutron wavelengths ($\approx$ Å) and energies ($\approx$ meV) match, respectively, the amplitudes and frequencies of molecular motions. Extremophiles and their macromolecules had to develop molecular mechanisms of adaptation to extreme physico-chemical conditions. Using neutron spectroscopy, we have measured molecular dynamics for in vitro soluble proteins adapted to extreme temperatures, as well as for in vivo macromolecules in living cells of psychrophile, mesophile, thermophile and hyperthermophile bacteria. We have demonstrated that molecular dynamics presents one of these molecular mechanisms of adaptation. Thermoadaptation appears to have been achieved by evolution through selection of appropriate rigidity, in order to preserve specific macromolecule structure, while allowing the conformational flexibility required for activity.

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molecular dynamics study of interaction of modeled RGS2 protein structure with lipids bilayer

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RGS2 protein belongs to the family of regulators of G-protein signaling (RGS) proteins.
We have shown that the N-terminal part of RGS2 protein can selectively modulate G protein signaling of CCK2 receptor. In addition it was reported in literature that N-terminal part has plasma membrane targeting activity. For structural studies of this complex protein-lipid interaction, the molecular model of RGS2 was built on the basis of combined approach including of homology modeling, circular dichroism spectroscopy, molecular docking and further was optimized and subjected to 10 ns duration MD-simulation in water-lipid (POPC) environment for studying interaction with lipid bilayer. The N-terminal part of RGS2 was initially set parallel to the lipid-solution interfacial region. During the simulation, the model was stable, helical part of N-terminal had an interaction with lipids in particular Lys34, 32, and Arg44, 33 bond with ester carbonyl group of the lipids and Leu37, 38, 45 and Trp41 penetrated into lipids. The results provide the RGS2-protein-lipid system which will be used for studying structural basis for CCK2R.RGS2 recognition.

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Ligand transfer dynamics in cytochrome c oxidase using infrared femtosecond spectroscopy

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Cytochrome *c* oxidase (CcO) couples electron and proton uptake associated with oxygen reduction to proton translocation across the membrane. Oxygen is reduced in a catalytic site containing a heme a_3 and a copper atom (Cu_B). The latter also acts as a "gate" for the entrance and exit of ligands to and from the active site.
We investigate the transfer of CO from the heme Fe to Cu_B after dissociation in beef heart CcO using visible pump – mid-infrared probe experiments with 300-fs resolution. The visible pump triggers the photodissociation of CO, thus initiating the transfer of the ligand from the heme Fe to Cu_B in <1 ps [1]. The infrared signature of the ligand transfer is the shift of the CO stretching frequency from 1963 cm^{-1} (CO bound to Fe) to 2062 cm^{-1} (CO bound to Cu_B) and an absorption strength decrease. The vibrational frequency and absorption strength change with time constants of about 200 and 350 fs, respectively. As in myoglobin [2], the vibrational frequency changes faster than the absorption strength. These results agree with molecular dynamics simulations showing that CO occupies a favorable position for binding to Cu_B after 300-400 fs [3]. This ultrafast transfer time from the heme to Cu_B points to an optimized pathway for efficient CO evacuation from the active site.
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The cooperative effect of phalloidin and jasplakinolide on the dynamic properties of actin filaments

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The effect of phalloidin and jasplakinolide on the dynamics and thermal stability of actin filaments was studied by biophysical methods. Temperature dependent fluorescence resonance energy transfer measurements showed that filaments of Ca-actin became more rigid in the presence of toxins. The differential scanning calorimetric (DSC) results also indicated that the stiffer filaments had greater thermal stability. Both techniques proved that the effect of jasplakinolide was greater than the effect of phalloidin. The DSC measurements showed that Mg-actin filaments could adapt three conformations in the presence of the drugs. One conformation was adapted in direct interaction with the drug, while another conformation was identical to that observed in the absence of drugs. A third conformation was induced through allosteric inter-protomer interactions. The effect of both drugs propagated cooperatively along the actin filaments. The number of the cooperative units determined by using a quantitative model was larger for jasplakinolide than for phalloidin. The cooperativity along the actin filaments can serve *in vivo* as an information channel allowing the different effectors to express their full effect even under substoichiometric binding conditions for better economy and less energy consumption of living cells.

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The cooperative effect of phalloidin and jasplakinolide on the dynamic properties of actin filaments

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Specific X-ray radiation damage and protein dynamics

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Intense synchrotron radiation produces specific structural and chemical damage to crystalline proteins even at 100 K. Disulfide bonds form radicals or break, acidic residues are decarboxylated and enzymatic active sites appear to be particularly radiation-sensitive. Specific radiation damage reflects the flexibility of sensitive parts in proteins and, therefore, can serve as a tool to study structural dynamics as a function of cryo-temperature. In this context, the physical state of the crystal solvent plays an important role since it changes as a function of temperature. Amorphous at 100 K, the solvent undergoes a glass transition and crystallizes upon warming. The change in solvent mobility at the glass transition leads to increased flexibility in the protein molecules, allowing radiation-induced structural changes to occur that cannot be observed at 100 K.
We studied the temperature-dependence of specific X-ray radiation damage to crystalline trypsin by protein crystallography and online microspectrophotometry at temperatures between 100 and 200 K. Above the solvent glass transition temperature in trypsin crystals (*i.e.* above 190 K), disulfide radical lifetimes drastically decrease and additional radiation-induced structural changes appear in the protein. Taken together, these results illustrate the relation among solvent and protein dynamics and specific radiation damage.

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Fishing the heme

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Several gram-negative bacteria use a Heme acquisition system involving an extracellular heme-binding protein HasA, also called hemophore. HasA hemophores are secreted in iron-limiting conditions by an ABC transporter. Their function is to acquire free or hemoprotein-bound heme and to deliver it to a specific outer membrane receptor. The *Serratia marcescens* hemophore, $HasA_{SM}$, is a 19-kDa monomer that binds one *b* heme with a strong affinity (Ka= 5.3 $10^{10}M^{-1}$). The three dimensional structure of $HasA_{SM}$ was determined both by X-ray crystallography for the holo-protein and by NMR for the apo-protein. The heme is highly exposed to solvent and it is held by two loops, each containing one of the axial ligands, His31 or Tyr74. The structures of the apo and holo-proteins show the same original $\alpha+\beta$ fold but large differences in the conformation of the heme-binding site. Indeed, the loop containing His31 moves by up to 20 Å in the apo form. ^{15}N Backbone dynamics were also studied by NMR for both forms. Apo- and holo $HasA_{SM}$ appear to be overall rigid proteins. But, the shifted loop (His31) displays fast motions on the ps-ns timescale, while the β-sheet, involving in the interaction with the receptor, exhibits conformational exchanges on the μs-ms timescale. The results obtained by NMR on the structure and dynamics of holo- and apo$HasA_{SM}$ are then discussed in terms of heme uptake and of heme transfer to the receptor.

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Femtosecond decay associated spectra of tryptophan in proteins: water relaxation or heterogeneity?

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We have obtained the complete (200fs-20ns) time- and wavelength-resolved fluorescence decay surface of Trp in the proteins Monellin and IIA^{Glc}, using both upconversion (for 200fs-200ps) and TCSPC (for 100ps to 20ns). Monellin contains 7 Tyrs and a single Trp. The E21W mutant of IIA^{Glc} contains a single Trp and no Tyrs, and that Trp is somewhat more solvent-exposed. We used a spinning multicell disk sample holder to avoid photodegradation effects. Both proteins exhibit complex decay kinetics. In addition to bulk water relaxation of $\sim$1.2ps seen in Trp and in both proteins, a 16ps decay component was found **only** in Monellin, with **positive** (preexponential) amplitudes even at long wavelengths (ca. 390nm). Usually, solvent relaxation yields a negative amplitude in the low energy wing of the spectrum. We suggest that this positive DAS (Decay-Associated Spectrum, the amplitude spectrum of a decay constant) originates from a highly quenched Trp conformer. Prior work (*PNAS*, **2002**, 99, 10964) ascribed the 16ps term to solvent relaxation via an exchange process between surface-bound water and the bulk. TRES evolution analysis will be shown. QM-MM simulations of the monellin relaxation (revealing only a picosecond solvent response) and simulations of candidate quenching processes will be presented.

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Different structural/functional contribution of three groups of tyrosine residues to the green plant MSP

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In soluble green plant manganese stabilizing protein (MSP), three groups of Tyr could be differentiated by NAI acetylation: approximately 5 Tyr could be easily acetylated (superficial), 1-2 could be acetylated when NAI concentration was sufficiently high (superficially buried) and other 1-2 could only be acetylated in the presence of urea (deeply buried). Acetylation of the 5 Tyr did not affect reconstitution, oxygen-evolving activities, and native secondary structure of MSP. Further modification of the 1-2 superficially buried Tyr completely abrogated the MSP rebinding and oxygen evolution activities. At least one tyrosine was inaccessible to NAI until MSP was completely unfolded by 8 M urea. A prominent red shift in fluorescence spectra of MSP was observed after modification of 6 Tyr, indicating a great loss of native secondary structure. CD revealed that MSP was mostly unfolded when 6 Tyr were modified and completely unfolded when all 8 Tyr were modified. Fluorescence and CD studies revealed that loss of MSP rebinding to PSII membranes following NAI modification correlated well with conformational changes in MSP. Together, our results indicate that different tyrosine residues have different contributions to the binding and assembly of MSP into PSII.

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Molecular and structural insight into the DNA damage recognition complex of the NER

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Xeroderma pigmentosum (XP) is a hereditary disease characterized by photosensitivity, a high incidence of sunlight-induced skin cancer. XP patients can be classified into seven different genetic complementation groups (XPA to XPG), all of which are defective in NER, a highly conserved and efficient mechanism for the elimination of DNA damages. The XPC complex including the proteins XPC, hHR23B, and HsCen2, plays a critical role in the first step of the NER process by detecting the damaged DNA, and recruiting other proteins necessary for the repair. HsCen2 is a calcium binding protein from the EF-hand superfamily, and is involved in other regulatory pathways, like the control of the cell division or of the mRNA export. The structures of the complexes formed by both HsCen2 and its C-terminal domain with the centrin binding peptide N847-R863 derived from the XPC sequence, determined by NMR spectroscopy, molecular dynamics and molecular modeling, will be presented. Only the C-terminal half of the protein is necessary for the peptide binding, while the N-terminal half keeps a closed conformation. Calcium titration of the protein/peptide complex revealed that the interaction may take place in the absence of metal ions and revealed a high plasticity of he human centrin. A larger fragment of XPC (815-940), including the centrin binding peptide conserves the interaction properties, but shows a very flexible solution structure.

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– Protein Reactivity and Dynamics –

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Protein competitive adsorption studied with imaging ellipsometry and atomic force microscopy

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Protein adsorption and competitive adsorption are important in many phenomena such as cell adhesion and proliferation and biocompatibility of biomaterials, thus being basic issue in areas such as tissue engineering and biomedical engineering. The adsorption and competitive adsorption of collagen, a kind of extracellular matrix proteins, and bovine serum albumin (BSA) on chemically modified silicon surfaces were directly visualized and quantified imaging ellipsometry and Atomic Force Microscopy (AFM). It was demonstrated that collagen and BSA in single component solution adsorbed on hydrophobic surface 2 times more than that on hydrophilic surface. The competitive adsorption between collagen and BSA showed that serum albumin preferentially adsorbed onto hydrophobic surfaces, but collagen on hydrophilic surfaces. In the binary solution of BSA (1 mg/ml BSA) and collagen (0.1 mg/ml), nearly 100% of the protein adsorbed on hydrophobic surface was BSA, but hardly adsorbed on hydrophilic surface. Surface affinity was the main factor controlling the competitive adsorption. Langmuir theory was used to deduce the kinetics of collagen and BSA adsorption, which fitted the experiments quite well. Based on the protein competitive adsorption, micro-patterned surface with hydrophilic/hydrophobic region was prepared which resulted in the patterning adsorption of collagen and BSA.

P-194

High-resolution proton NMR of membrane peptides

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Solid-state NMR of abundant protons is a challenging task due to the large homonuclear dipole-dipole couplings, which broaden the resonances and obscure the isotropic chemical shift assignment. Combining fast spatial rotation and rf rotation, a better proton resolution may be achieved for fast MAS NMR, but those methods are not applicable to most biological systems, such as membrane peptides and proteins.
Conventional CRAMPS NMR has been applied to crystallized amino acids, peptides and powdered model compounds, but nothing has been reported on real biological systems to study function related conformational dynamics. A new approach is proposed here in which sample orientation is constrained and homonuclear decoupling is used to study membrane peptides and proteins at a low MAS frequency. The new method is named as Combined Oriented Sample Rotation And Multiple-Pulse Spectroscopy (COSRAMPS) by analogy to the CRAMPS experiments. High-resolution proton spectra have been achieved in 1D and 2D COSRAMPS experiments on two oriented membrane peptides and proteins to study structural dynamics in membrane bilayers.

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Crystallographic analysis of peptide deformylase from Leptospira interrogans

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Peptide deformylase is an attractive target for developing novel antibiotics. Here, we report a series of native and inhibitor-complexed structures of *Leptospira interrogans* PDF (LiPDF). The existence of a "closed" substrate-binding pocket in the free enzyme, which was proved to be active, was repeatedly confirmed by crystallization under different conditions and crystal packing analysis. In the complex structure, the competitive inhibitor actinonin was observed in a "half-open" substrate-binding pocket. The key switch loop (CD-loop), which causes the "closed (inactive)" and the "open (active)" substates of LiPDF was observed to change conformations with Cá shifts of up to 6Å. The active enzyme with a closed substrate binding pocket seems to be impossible in a traditional view but could be interpreted by a "population shift" model. These results, for the first time via crystallography, suggest the functionally important conformational selection by substrate binding, and also remind us that the structure we observed could be only the major population, but the minor population we didn't observe could also have important biological significance.
Keywords: peptide deformylase, conformational changes, population shift, crystal structure

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Cooperative binding of the transcriptional repressor CggR to the gap A promoter modulated by FBP

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One of the main catabolic pathways in *Bacillus subtillis* is glycolysis. The enzymes that catalyze the central steps of glycolysis are encoded by the gap A operon, whose regulation at the transcriptional level is achieved by the repressor CggR. Fluorescence anisotropy experiments have shown that the binding of CggR to its DNA target is very cooperative at 150 mM NaCl. At this NaCl concentration CggR binds specifically to the labelled DNA while lowering the NaCl concentration to 50 mM results in significant non-specific binding. The cooperativity of CggR binding to DNA decreases dramatically upon the addition of fructose 1,6 biphosphate (FBP). The effect of FBP on CggR binding to the DNA increases with its concentration up to 2 mM, being noticeable at concentrations as low as 0.01 mM. Preliminary results indicate that the binding stoichiometry might be different in the presence of FBP. The stoichiometry of binding of CggR to the target DNA, both in the presence and absence of FBP, is investigated by stoichiometric binding titrations in conjunction with analytical ultracentrifugation experiments. Parallel experiments on the binding of the gap B repressor, CcpN, to its DNA targets will provide a global understanding of the transcriptional regulation of the gap A and gap B operons in *Bacillus subtillis*.

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– From DNA to Chromatin –

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Thermodynamic of (CNG)n trinucleotide repeats implicated in trinucleotide repeat expansion diseases

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Trinucleotide repeats are involved in a number of debilitating diseases such as myotonic dystrophy, Huntington disease and fragile X syndrome. 12 to 75 base-long (CNG)n N=A,T,C,G oligodeoxynucleotides were analysed using a combination of biophysical (UV-absorbance, CD, differential scanning calorimetry) and biochemical methods (non denaturing gel electrophoresis, enzymatic footprinting). All oligomers formed intramolecular structures with a melting temperature which was only weakly dependent on oligomer length. All sequences form stable structures at 37°C under near physiological conditions. Thermodynamic analysis of the denaturation process by UV-melting and calorimetric experiments revealed a surprising length-dependent discrepancy between the enthalpy values deduced from model-dependent (UV-melting) and model-independent experiments (Calorimetry). Evidence for non-zero molar heat capacity changes was also derived from the analysis of the Arrhenius plots and DSC profiles. Such behavior is analyzed in the framework of a polyhairpin model, in which long CNG oligomers do not fold into a simple long hairpin-stem intramolecular structure, but allow the formation of several independent folding units of unequal stability.

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The relations between GSTM1 polymorphism and human sperm cell oxidative damage

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This study has been performed to determine the relation between Glutahtione S- transferase polymorphism and idiopathic infertility in men. After isolation of genomic DNA from leukocytes, to determine the GSTM1 polymorphism PCR analysis has been performed. According to PCR results both patient and control group inviduals were divided into two different groups according to their GSTM1 gene structure as GSTM1 null and GSTM1 positive. And named patient GSTM1 null as group 1(G1), GSTM1 positive as group 2 (G2), control GSTM1 null as group 3 (G3) and control GSTM1 positive as group 4 (G4).When MDA and protein oxidation values were compared, it has been detected that as a product of oxidative stress statistically significant alterations were also seemed to be ROS dependent. As a conclusion of present study it may be said that:Presence of infertility in the condition that GSTM1 gene is (+) in patient group, shows that GSTM1 gene is not the only gene that responsible for infertility in men. Higher ROS levels in both sperm cells and seminal plasma of GSTM1 (-) infertile individuals may be refer that oxidative stress is the reason of GSTM1 gene polymorphism in infertility.It may be said that one of the important reason of infertility in infertile individuals is the GSTM1 (-) gene polymorphism.

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Compaction of single-chain DNA by histone-inspired nanoparticles

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The complex of DNA with a cationic octamer of core histones is the elemental unit of chromatin which allows for a large-scale compaction while preserving gene activity. We elaborated a versatile experimental model of chromatin which consists of a single chain of long DNA that interacts with synthetic cationic nanoparticles of various sizes. The DNA/nanoparticle interaction was characterized by direct single-molecular observations in the bulk solution by fluorescence microscopy and the nanostructure of the DNA/nanoparticle complexes was resolved by transmission electron microscopy. We found that the single-chain DNA compaction by nanoparticles is stepwise and progressive. It proceeds through the formation of beads-on-a-string structures, the DNA chain wrapping around individual nanoparticles. We investigated the effect of particle size and salt concentration on compaction efficiency and mechanism of wrapping. Notably, the DNA compaction is optimal at a physiological salt concentration and complexed nanoparticles are overcharged by DNA.

P-200

Torsional manipulation of single chromatin fibers reveals a highly flexible structure

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Magnetic tweezers (1) were used to study the mechanical response under force and torsion of single nucleosome arrays assembled on periodically repeated positioning sequences. Chromatin fibers were found to be torsionally softer than naked DNA, being able to accommodate a large amount of supercoiling at nearly constant length (2). This behavior is described by a quantitative model of chromatin 3D architecture (3), based on the existence of three possible topological states of the nucleosome (4). Under torsional strain, nucleosomes can transit between these states and the fiber undergoes considerable reorganization of its internal conformation, that depends on the amplitude and sign of supercoiling. Moreover, the rotational flexibility appears to strongly depend on the regularity of the nucleosome spacing along the fiber. The relevance of the results for *in vivo* processes such as transcription and chromatin remodeling is discussed.

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Posters
– From DNA to Chromatin –

P-201

Electrostatic effects on interaction and conformation of nucleosome core particles

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The eukaryotic chromatin is densely packed within the nucleus. The local chromatin organization and the processes leading to its dense packing are still puzzling. We aim at understanding the role of local charges and ionic concentrations variations on the supramolecular chromatin organization.
We have focused our studies on the repeated chromatin units, which are the nucleosome core particles (NCPs). They are made of 146 DNA base pairs wrapped around a histone octamer. Those colloidal particles are heterogeneously charged with negatively charged DNA and mainly positively charged histones.
We prepare NCPs either from calf thymus chromatin or from recombinant DNA and histones. In order to estimate the role of histone tails and to vary the particles charges, recombinant NCPs are reconstituted with different tail combinations. Interactions between NCPs and their conformation are assessed using Small Angle X-rays scattering experiments with varying ionic conditions. We have demonstrated that tails are necessary for having attractive interactions between NCPs (1). Dense phases obtained with monovalent or divalent ions are analysed using freeze fracture and cryo-electron microscopy.
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P-202

Interaction of the alkaloid berberine with B-form, HL-form, and protonated structures of DNA

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DNA is a structurally polymorphic macromolecule that can adopt surprising range of structures *in vitro*. The protonation induced conformational changes in homo and hetero GC polymers and natural DNAs were studied using spectrophotometric, thermal melting and circular dichroic techniques. At pH 3.4, 10 mM [Na^+] and at low temperature, the synthetic and natural DNA's adopted a unique and stable structure different from their respective B-forms and characterized to have specific absorption, CD and Tm characteristics. The protonated structure of the hetero-polymer of G.C has been established to be left handed with Hoogsteen base pairing (H^L–form). The nature of the protonated structure was further investigated by monitoring the interaction of berberine. The binding of berberine resulted intrinsic circular dichroic changes with the generation of induced circular dichroic positive bands. Extrinsic circular dichroic studies showed the generation of circular dichroic bands with opposite sign and magnitude compared to their B-form structure. The alkaloid bound to both the forms in a non-cooperatively. Fluorescence studies showed remarkable increase of the rather weak intrinsic fluorescence of berberine on binding to protonated structures compared to the B-form structures in all cases. These results possibly indicate different stacking arrangement of berberine at the interaction site of B-DNA, H^L-DNA and protonated DNA structure in synthetic and natural DNAs that potentiates its use in regulatory roles for biological functions.

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The investigations of DNA-modified by cis-DDP with nuclear proteins HMGB1 and HMGB2

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Cisplatin (cis-DDP) is one of the most successful anti tumor drugs. The biological activity of cisplatin is based on its ability to form stabile adducts with DNA. Cis-DDP binding changes the structure of DNA and prohibits its proper functioning in living cell. Some chromatin proteins (e.g. HMGB proteins) can bind specifically to cis-DDP-DNA addicts. HMGB1 and HMGB2 proteins are the members of a super family of High Mobility Group proteins. Although these proteins are very similar (80% homology) the both proteins are present in the cell. The difference in the proteins' functional role is not clear yet. Earlier we showed that the secondary structures of HMGB1 and HMGB2 proteins are slightly different, so we could expect that they interact with DNA in different manners.
We have studied the formation of cis-DDP adducts using circular dichroism spectroscopy. We have shown that the structures of HMGB1 and HMGB2 proteins changes differently upon the interaction with DNA. The properties of DNA circular dichroism in complexes with HMGB1 are determined by the interaction of DNA with cisDDP. In the case of HMGB2 increasing protein content leads to the shift of CD spectra towards the longer wavelengths while its intensity grows. There are two possible reasons for the distortions in DNA structure. First, HMGB2 may interact not only with adduct sites on DNA but also with platinum-free regions; and/or, second, HMGB2 has higher affinity to DNA damaged by cis-DDP, compared to the affinity of HMGB1. Both of these factors would lead to the considerable distortion in DNA structure.

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The two-angle model and the phase diagram for Chromatin

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We have studied the phase diagram for chromatin within the framework of the two-angle model. Rather than improving existing models with finer details our main focus of the work is getting mathematically rigorous results on the structure, especially on the excluded volume effects and the effects on the energy due to the long-range forces and their screening. Thus we present a phase diagram for the allowed conformations and the Coulomb energies.

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– From DNA to Chromatin –

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Conformational dynamics of single-stranded DNA

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We present measurements of conformational changes in single-stranded DNA molecules monitored through fluorescence quenching of organic fluorophores. Guanosine efficiently quenches fluorescence of oxazine derivatives via photoinduced electron transfer. We used steady-state and time-resolved fluorescence spectroscopy, as well as fluorescence correlation spectroscopy (FCS) with nanosecond time-resolution, to investigate the interaction between guanosine and the oxazine fluorophore MR121 in aqueous solution. We demonstrate that quenching occurs primarily upon formation of ground-state complexes between MR121 and guanosine.
To probe conformational dynamics of short single-stranded DNA (ssDNA) molecules, we attached MR121 to the 5'-end of ssDNA consisting of 1-10 thymine residues and a single guanine residue at the 3'-end. We then monitored equilibrium fluctuations in the fluorescence signal using FCS. Fluctuations are caused by fluorescence quenching upon intramolecular complex formation of guanine and the attached fluorophore, and directly report on end-to-end contacts. We determined end-to-end contact rates for ssDNA on a length scale that is inaccessible by monitoring Förster resonance energy transfer (FRET). Our results reveal a power-law dependence between contact rates and the number of nucleotides that is in agreement with predictions by polymer theory.

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DNA adsorption and renaturation on functionalized surfaces

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A recent study has shown that a specific interaction exists between single-stranded DNA and phenol, increasing the kinetic of DNA renaturation at the interface water-phenol interface by a factor of one million. (1)
To better understand the mechanisms of this interfacial reaction, phenolic or aminated groups are grafted onto solid substrates. It allows possible applications for direct DNA imaging and for improving hybridization techniques which are explored here. In a first step, atomically-flat silicon (111) surfaces (2) are functionalized by wet chemical approaches (3). The AFM images show that surfaces are perfectly defined on the nanometer scale and the corresponding ATR-FTIR spectra quantitatively characterize the modified surfaces.
The next step is to adsorb a single-stranded DNA chain onto the functionalized surfaces, to study its conformation by AFM imaging and to understand electrostatic and aromatic interactions in the adsorption process. Then, by adding the complementary strand, one can try to mimic the water-phenol interfacial renaturation.
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P-207

Specificity in dna recognition by peptide from papillomavirus e2 protein

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The E2 proteins of papillomavirus specifically bind to double-stranded DNA containing the consensus sequence ACCG-N4-CGGT, where N is any nucleotide. Here, we investigate the ability of an 18-aminoacid peptide (alpha1E2), corresponding to the DNA-recognition helix, alpha-helix-1, to bind and recognize dissimilar DNA sequences. DNA binding assays performed with consensus sequence show saturable curves with alpha1E2 peptide, and the alpha1E2 peptide is converted to an ordered conformation upon complexation. Isothermal binding assays performed with a non-specific DNA lack saturating levels, which is characteristic behavior of non-specific binding. Alpha1E2 peptide binding to non-specific DNA presents much larger dependence on counterion concentration than its binding to specific one, indicating a different, sequence-dependent mechanism of interaction. Quantitative stoichiometric analysis revealed that alpha1E2 peptide displays full capability for ACCG site recognition. To our knowledge, this is the first report where DNA binding and nucleic acid bases discrimination by a peptide is demonstrated. Taken together these results reveal that the majority of the specificity determinants reside in the peptide, and that a minimal amino acid sequence is sufficient to drive specificity.

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How Elastic Are Biopolymers? Mechanical Properties of DNA

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The Young's dynamical modulus ($\boldsymbol{E}$) and the logarithmic decrement of damping ($\grave{\boldsymbol{e}}$) of the DNA films are measured at frequencies from 50 Hz to 20 kHz in the temperature range from 300 to 100^{o}K. It is shown that the Young's modulus of wet DNA films strongly increases with decreasing hydration. Dependence of $\boldsymbol{E}$ and $\grave{\boldsymbol{e}}$ on hydration is of a complex character. Young's modulus of denatured DNA films is larger that of native ones. All peculiarities of changing of Young's modulus and logarithmic decrement of damping of native DNA films vanish in case of denatured ones. The native and denatured DNA isotherms are different. The Young's modulus of DNA films containing $\geq 0,7 gH_2O/gdryDNA$ is found to decrease with increasing temperature, undergoing a number of step-like changes accompanied by changes in film hydration. At low water content changing of $\boldsymbol{E}$ with temperature increasing takes place smoothly. A phase transition of order-disorder type is discovered in the temperature range from 240 to 160 ^{o}K which is accompanied by a several orders of magnitude increase in the Young's modulus. The logarithmic decrement of damping has several maxima in the range of phase transition. The dependence of this transition on the hydration level and conformation state of DNA is studied.

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The comparison of different calculation methods of the melting temperatures for DNA-ligand complexes

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The melting curves of double helical polynucleotides-ligand complexes shift to the high or low temperature area as compared with the melting curve of "pure" polynucleotides. Ligands may stabilize or destasbilize native DNA. The shift (ΔT_m) of the melting temperature of DNA-ligand complexes (T_m) compared with melting temperature of "pure" DNA (T_0) may be estimated from the Tm concentration dependencies by different methods. Nevertheless, these approaches have not taken into account the different binding modes. The temperature dependencies of binding constants have not taken into account also. The complexes of actinocin derivative (Act II) with some polynucleotide matrixes: DNA from Micrococcus lysodeikticus (∼72% GC), Clostridium perfringers (∼28% GC), DNA from calf thymus (∼48% GC), poly2(rC) (pH = 4.4) were investigated. The complexes of 6-azacytidine (6-AZC) with calf thymus DNA and DNA samples extracted from epididymis of Wistar male rats exposed to chronic γ-radiation were investigated. The temperature dependencies of absorption spectra of double helical polynucleotides–ligands mixtures were used to determine the thermodynamic parameters of binding : ΔH, ΔS, ΔG values by Vant Hoff equation and Marky's approach. Different approaches for determining ΔT_m (equations of Frank-Kamenetskii, McGhee, Marky) are discussed. The approaches from theory of chemical equilibria for estimation of ΔT_m taking into account the temperature dependencies of binding constants are discussed also.

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DNA structural transitions induced by divament metal ions in aqueous solutions

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Using methods of IR spectroscopy, light scattering, gel-electrophoresis DNA structural transitions are studied under the action of Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+} ions in aqueous solution. Upon interaction with divalent metal ions studied DNA undergoes structural transition into a compact form. The DNA secondary structure in condensed particles corresponds to the B-form family. The mechanism of DNA compaction under Mt^{2+} ion action is not dominated by electrostatics. The effectiveness of the divalent metal ions studied to induce DNA compaction correlates with the affinity of these ions for DNA nucleic bases. Mt^{2+} ion interaction with DNA bases may be responsible for DNA compaction.

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VCD spectroscopy of DNA-drug interactions: DNA-AZT

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Vibrational circular dichroism (VCD) is a chiraloptic technique, which has proven useful to examine the effects of various agents on DNA structure. In our laboratory we have used this technique to investigate DNA interactions with several metal ions such as Ca^{2+}, Mn^{2+}, Cr^{3+} and Pt^{2+} (1-3). Among several different types of DNA-drug interactions, netropsin is a known DNA minor groove binder while methyl green is a common DNA major groove binder (4,5). Daunomycin and ethidium bromide intercalate into DNA (6,7). 3'-Azido-3'-deoxythymidine (AZT) is a drug commonly used for treating HIV-1 infections. It is believed that AZT is converted into 3'-azido-3'-deoxythymidine-5'-triphosphate and incorporated into DNA where it inhibits DNA replication by chain termination (5). We are currently investigating the interaction of DNA with AZT by various spectroscopic methods including VCD and electronic CD. The results of this study will be reported.

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RNA synthesis during transcription initiation occurs through a DNA-scrunching mechanism

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Transcription by RNA polymerase (RNAP) is a multi-step process central to gene regulation; much of the regulation occurs before and during transcription initiation, i.e., before RNAP breaks its contacts with promoter DNA. However, the mechanism of initial transcription, also known as abortive initiation (since it involves iterative synthesis and release of short RNA, 2-9 nt in length), is currently unresolved. We studied abortive initiation by monitoring the stoichiometry and conformation of transcription complexes using single-molecule spectroscopy in solution. We established that most transcription complexes were active in abortive initiation and promoter escape. Under the same conditions, we observed DNA compaction ("DNA scrunching") during abortive-RNA synthesis. This compaction involved relative movement of downstream DNA towards RNAP, and no movement of upstream DNA relative to RNAP; the extent of compaction was proportional to the length of abortive RNA synthesized, and was abolished by a drug that prevents abortive initiation. Our measurements suggest a pure DNA-scrunching mechanism for abortive initiation, where downstream DNA is "reeled" in and out of the main RNAP channel during each cycle of abortive-RNA synthesis. Our results have important implications for transcription start-site selection and promoter escape.

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Interrelated protein and DNA structural changes upon TATA box binding by TBP(TATA binding protein)

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The TATA Binding Protein (TBP) participates in the assembly of transcription pre-initiation complex (PIC) although the role of DNA binding by TBP is uncertain.
Using intrinsic tyrosine and tryptophan fluorescence of TBP and its conserved C-terminal core domain (TBPc) to separately monitor the two domains of the protein, together with fluorescence resonance energy migration of end-labeled 14 bp DNA bearing the TATA Box sequence TATAAAAG to follow the DNA conformational change, we have shown that both macromolecules change conformation upon their interaction. TBPc has a more compact structure than the C-terminal domain within the full-length protein [S. Khrapunov, N. Pastor & M. Brenowitz, (2002) *Biochemistry*, 41, 9559]. Analysis of fluorescence quenching by DNA and external quenchers reveals that the N-terminal domain unfolds and changes its position relatively to the C-terminal domain upon DNA binding. In addition, the N-terminal domain partially shields the 'DNA binding saddle' of the C-terminal domain that interacts with DNA. These data support an auto-inhibitory mechanism in which competition between the N-terminal domain and DNA for the saddle diminishes the DNA-binding affinity of the full-length protein [Rashidzadeh, H., Khrapunov, S., Chance, M.& Brenowitz, M. (2003) *Biochemistry* 42, 3655].

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Topology and geometry of DNA target-site alignment in the Flp and Cre synaptic complexes

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The Flp recombinase of yeast and the Cre recombinase of bacteriophage P1 both belong to the lambda-integrase (int) family of site-specifc recombinases. With wild-type recombination sites the exclusive directionality of intramolecular recombination depends strictly on the relative orientation of target sites on a DNA molecule. This implies that productive recombination reactions require a particular geometric relationship between target sites at synapsis. We have used atomic-force microscopy to investigate the topology of Flp and Cre recombination taking place on circular DNA molecules. An examination of the tertiary structure of synaptic complexes formed on open-circular substrate DNAs, in conjunction with topological analysis using the mathematics of tangles, shows that only a limited number of topologies are consistent with the global alignment of recombination sites. These topological solutions, along with information about the chirality of recombination, support a common model for the recombination pathways that accounts for many of the available experimental data on these systems.
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Loop-closure analysis of DNA site synapsis in tyrosine recombinases

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We investigate the kinetics of intramolecular recombination by the Flp and Cre recombinases using closely spaced recombination sites. The contour separation of directly-repeated sites in these constructs is on the order of the persistence length of the DNA duplex, requiring the formation of small DNA loops during synapsis. Kinetics of recombination examined at incremental site spacings over nearly two helical turns are dramatically affected by elastic deformation of the intervening DNA during loop formation and hence extremely sensitive to the geometry of recombination sites at synapsis. Using a new theory for the helical-phase dependence of DNA looping, we show that preliminary recombination data are consistent with a site synapse involving a nearly perfect right-angle crossing of target sites. The solution structures are neither parallel nor antiparallel and are also inconsistent with the structural parameters obtained from high-resolution crystallographic structures of the recombinase-DNA complexes.
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Energy barriers and rates of tautomeric transitions in DNA bases: A quantum chemical analysis

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Tautomeric transitions of DNA bases are proton transfer reactions, which are important in biology. These reactions are involved in spontaneous point mutations of the genetic material. Intrinsic Reaction Coordinates (IRC) analyses through a*b initio* quantum chemical calculations are carried out for the individual DNA bases A, T, G, C and also A:T and G:C base pairs to estimate the kinetic and thermodynamic barriers for tautomeric transitions. Relatively higher values of kinetic barriers (about 50-60 kcal/mol) for the single bases indicate that tautomeric alterations of isolated single bases are quite unlikely. On the other hand, relatively lower values of the kinetic barriers (about 20-25 kcal/mol) for the DNA base pairs A:T and G:C clearly suggest that the tautomeric shifts are much more favorable in DNA base pairs than in isolated single bases. The unusual base pairing A':C, T':G, C':A or G':T in the daughter DNA molecule, resulting from a parent DNA molecule with tautomeric shifts, is found to be stable enough to result in a mutation. The transition rate constants for the single DNA bases in addition to the base pairs are also calculated by computing the free energy differences between the transition states and the reactants.

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Order in the high density solutions of nucleosomal core particles

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We investigate different types of order in the high density solutions of nucleosomal core particles (NCP). Digestion of the linker DNA between nucleosomes along the eucaryotic genome gives rise to the NCP with a DNA fragment wrapped around the histone. At high enough density the solution of NCPs undergoes a sequence of phase transitions. Under change of monovalent salt concentration and osmotic pressure NCPs aggregate into columns. Due to the interaction between the NCPs these columns successively form a lamellar, 2D hexagonal, 2D inverted hexagonal and 3D hexagonal phases. We propose a unified mechanism for all transitions in the high density NCP solutions. It is related to a high shape and charge asymmetry of the NCP due to the DNA wrapping around the histone core. The entry-exit point of the DNA free ends marks a dyadic axis making the asymmetry visible on cryoelectron micrographs. We show that hexagonal-to-lamellar transition is driven by the periodic antiparallel vector field and results in simultaneous dyadic axes correlations along the column and between the columns, and in the correlated displacement of columns. Detailed analysis of the mechanism allows us to predict other ordered phases. Calculated phase diagrams help to understand the relative stability of different NCP arrangements in condensed solutions.

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Supermolecular organization in high density DNA solutions

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Condensed solutions of DNA fragments in a biologically relevant range of densities undergo the sequence of phase transitions : cholesteric liquid crystal (LC) phase - hexatic and distorted hexatic LC phase - orthorhombic crystalline phase. Some of these mesophases are similar to the DNA organization chosen by starved *E.coli* for its survival strategy. We propose a model which describes the whole phase sequence. It accounts for angular frustrations between neighboring DNAs induced by electrostatic interaction between parallel helical macromolecules covered with adsorbed counterions. The stability of the phases in the high density region is analyzed. The peculiarities of organization evidenced by x-ray scattering in osmotically condensed DNA are discussed. The relation between the spontaneous elastic distortion of a liquid crystal phase and the elasticity of individual DNA molecules is also analyzed. We show that the major part of the distortion energy expelled from the LC medium is accumulated by individual DNA molecules. The resulting process is a DNA helix overwinding, observed experimentally in the high density region. We estimate typical values for the free energy of the overtwisted state, and for the elastic torque, using the data of single DNA manipulation and discuss the probability of the overtwisted state in a crowded cell environment.

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DNA and RNA quadruplexes

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G-quadruplexes are unusual nucleic acids structures based on the association of planar G-quartets. G-quadruplexes can be classified according to the number of strands that self-associate and further differentiated by their relative orientations. Stable tetramolecular quadruplexes may be formed with short oligomers. The melting of these quadruplexes is kinetically irreversible, allowing us to study association and dissociation processes independently (1-2).
Quadruplexes may find applications in areas ranging from nanotechnology to medicinal chemistry. A nanomolecular machine has been designed based on a duplex-quadruplex equilibrium (3). The 3' G-rich telomeric overhang may adopt a G-quadruplex structure that blocks telomerase. We have identified by FRET several series of G4 ligands that also exhibit potent and specific anti-telomerase activity (4-5). Our data show that a G-quadruplex interacting agent is able to impair telomere function in a tumor cell line (5-6) thus providing a basis for the development of new anticancer drugs.
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P-220

Rearangement in the chromatin parts under influence of non-thermal electromagnetic radiation

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Gene function is subjected to the effects of surrounding chromatin. The nature of these effects may be epigenetic occurring in some cell, but under influence of some external factors these processes can be disrupted. Inside the nucleus there are three structural compaction of DNA. Regions of dense heterochromatin masses scattered throughout the interphase nucleus. Only recently has some understanding of the mechanisms of its formation and propagation been achieved. Heterochromatin is involvement in epigenetic silencing phenomena including repression along extended regions of chromosomes and the inactivation of whole chromosomes.
In the presented work we study changes in melting parameter of chromatin having different localization in nucleus: euchromatin and heterochromatin under influence of coherent non thermal electromagnetic irradiation on wheat seeds during germination. The EHF generator with the range of working frequencies 37,5-53,5 GHz was used as a source of monochromatic radiation of mm-waves. The irradiation was carried out in a distant zone of radiation of the generator.
Our experimental data allows us to expect that influence of coherent non thermal electromagnetic irradiation on wheat seeds during germination lead to the significant changes in some part of heterochromatin and in result to increasing of preservation system of living organisms.

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DNA structure al low pH values and in the presence of manganese(II) ions: a Raman study

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Raman spectra of calf thymus DNA were measured in the pH interval 6.4 to 3.45 in the presence of divalent manganese ions. pH-dependent protonation of AT and GC base pairs and conformational changes were indicated in the spectra. Protonation of adenine residues becomes obvious at pH 4.4 and continues upon lowering the pH to 3.45. Adenine protonation is connected with the disruption of AT base pairs. Protonation of GC base pairs is indicated at somewhat lower pH than that of AT base pairs, namely at pH 3.8, and continues upon lowering the pH to 3.45. At pH 3.8 unstacking of thymine residues is indicated, and spectral markers for the unstacking of adenine and cytosine were found at pH 3.45. Changes of the DNA backbone are indicated by spectral changes of conformational marker bands at 898 cm^{-1} and 1423 cm^{-1}.

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Accessibility and diffusion of transcription factor complexes into 1Mbp chromatin domains

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Presently for the experimentally observed chromosomal foci a rosette like structure of chromatin loops is assumed to exist. We developed a polymer model on the basis of the 30nm fiber which describes the folding in rosette like structures when we introduce attractive sites along the fiber. These attractive sites, representing condensing agents (HMG/SAR, HP1, cohesin, condensin, DNA-DNA interaction etc.), tend to aggregate and form rosettes. Presently there still two opinions about the aggregation process of transcription and splicing factors. They might form functional complexes directly at the sites of genes. Alternatively, (sub-) complexes are built up at distant sites, in the so called inter-chromatin regions, and subsequently reach the genes by passive diffusion. In the later case, such aggregates are in the order between 55 and 70nm in diameter and have to access the active genes to allow transcription. Brownian Dynamics simulations of rosettes/TF complexes were performed to investigate the dynamics of the interaction between the 30nm fibre and the proteine complexes. Quantities investigated are, e.g. penetration depth, average time spent in the core of the rosette and the density profile. Results are compared to experimental data of the steady state distribution of labeled NLS-strepavidin from dense chromatin regions and other nuclear structures.

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Electrostatic interactions with histone tails may bend linker DNA in chromatin

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A simple modeling of the electrostatic interactions mediated by salt concentration between linker DNA and histone tails in chromatin at physiological conditions is given. Linker DNA is represented as an elastic negatively charged line while histone tails are represented as one or more counter macroions, represented as spheres or curves of charge and radius modulated according to the participating tails.
It turns out that direct electrostatic interactions between macroions of opposite and high charge density and the Manning counterion condensation-release entropic mechanism, greatly bend linker DNA in opposition to DNA non electrostatic elastic force. Reasonable values of charge and radius of curvature of the counter macroions, representing one or more tails, may produce a DNA bending angle greater than 90°.
A simple model of dinucleosome is given that is condensed in physiological conditions due to bending of linker DNA. The bending decreases with salt concentration. This is in qualitative agreement with sedimentation and light scattering experiments on dinucleosomes, thus supporting the idea that bending of linker DNA may be one of the important mechanisms of condensation of oligonucleosomes with increasing salt concentration and in turn of the structure of the 30 nm chromatin fiber.

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DNA accessibility within nucleosome arrays

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DNA sites wrapped into chromatin *in vivo* are sterically occluded from proteins that must bind for vital biological processes to proceed such as RNA transcription, DNA replication and homologous recombination. The probability for a protein to bind a site within a single nucleosome has been determined (Polach and Widom, 1995; Anderson and Widom, 2000); however, it is not known how the accessibility changes for a DNA site within a chain of nucleosomes. We determined the equilibrium constant for site exposure of various unique restriction enzyme sites within di- and heptadecanucleosomes relative to single nucleosomes by the restriction enzyme kinetics assay. Nucleosome arrays were assembled by reconstituting 177 bp tandem repeats of a variant of the high affinity nucleosome positioning sequence 601 (Lowary and Widom, 1998) with purified core histone proteins, which has recently been shown to assemble into homogeneous nucleosome arrays (Dorigo, *et al*, 2004 and Huynh, *et al*, 2005). We find that DNA sites in a nucleosome, which is embedded within a dinucleosome or a heptadecanucleosome, has accessibilities similar to that of single nucleosomes. These results suggest that higher order chromatin structure does not contribute significantly to occluding proteins from DNA recognition sites within chromatin and that site exposure within chromatin is dominated by conformational fluctuations at the single nucleosome level.

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Spectroscopic and AFM studies of supramolecular order in complexes of DNA with HMGB-domain proteins

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HMGB-domain proteins constitute a family of non-histone chromosomal proteins known as 'architectural factors' of chromatin. Each protein in the family contains at least one structur-function DNA-binding domain called HMGB-domain. The family is known by members such as HMGB1, LEF-1, SRY, UBF and TCF-1. In addition to HMGB-domains, some of the proteins contain C-terminal sequences of Asp and Glu amino acid residues, which are believed to be modulators of the proteins' DNA binding activity. It was shown that the HMGB-domain sequence is able to form highly ordered complexes with DNA. Optical properties of such complexes are similar to those of DNA liquid crystals. Our present study aims to reveal structural organization of these DNA protein complexes. Using a combination of circular dichroism, fluorescence and atomic force microscopy we have shown that the structural organization of the complexes depends on ionic strength and protein to DNA ratio (***r***) in the complex, but most of all on the presence or absence the C-terminal acidic sequence. We have demonstrated that regardless of the presence of the Asp/Glu sequence the proteins induce remarkable DNA compaction. However, a protein lacking this C-terminal domain induces more considerable structural distortions in DNA and at some critical ***r*** induces the formation of highly ordered DNA-protein complexes revealed by AFM images.

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Chemical Modulation of Chromatin Gammalysis

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Gamma-photons are used in the therapy of various forms of cancer. Our study aims at understanding the modulatory effects of radioprotective compound Na-thyoglycholate during irradiation with 1,25MeV ã-photons used in usual cobaltotherapy, upon normal chromatin and upon carcinous tumor chromatin. Normal chromatin extracted from Wistar rat liver or carcinous chromatin from Walker tumors induced in Wistar rats was γ-irradiated with a Siemens ^{60}Co-source cobaltotherapy apparatus in the presence of the radioprotector.
The following parameters were analyzed:
•absorption spectra of the chromatin-ethydium bromide (EB) complexes at λ_{max}= 480 nm
•thermal transition spectrophotometric aspects
•the batochromic effect –the shift of the absorption maxima- of EB in chromatin complexes in either treatments.
As expected, γ-radiation produces both Single Strand Breaks (=SSB) and Double Strand Breaks (=DSB) in DNA and also nucleoprotein denaturing as indicated by intrinsic fluorescence.
The observed clastogenic effects upon DNA resulting in Strand Breaks are stronger on carcinous tumor chromatin than in normal chromatin and are proportional with the incident radiation dose.
The compound Na-thyoglycholate showed protective effects during γ-photons irradiation, upon both normal chromatin and carcinous tumor chromatin, in a chemical dose-dependent manner.

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Biophysical modeling of chromosomal aberration based genome instabilities

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A profound influence of the 3D spatial organisation of the genome on the genome instability and cancer related biological consequences yet to be well characterised. To mechanistically assess the implications of higher order chromosome geometry on inter and intra chromosomal exchanges, we calculated the radiation-induced aberrations by implementing the SCD model ('*Spherical 1-Mbp Chromatin Domain*', G. Kreth et al., 2001). Using a virtual irradiation algorithm on both lymphocyte and fibroblast nuclei models, the relative aberration frequencies depend on the assumed spatial distribution of chromosome territories were calculated. In aberration studies, we observed the dose-response regression curve exhibits a strong linear-quadratic fit and found to be in better accordance with experimental data. Besides, the genome level instability for variety of functionally significant lymphomas were measured for calculated at low LET gamma doses ranging from 0 to 5 Gy. In particular, we calculated possible exchanges between cancer gene specific break point region exchange with 200000 nuclei configurations. Interestingly, we observed our virtual nuclei configurations shown no lymphoma related exchanges at higher nuclei configurations suggesting the exchange event most unlikely to be random in cells.

P-228

Local dynamics of DNA. A time resolved fluorescence study of 2-aminopurine dinucelotides

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DNA dynamics on the scale of individual bases plays an essential role in a range of phenomena such as DNA-protein interaction [Roy *et al*, EMBO J '04] and electron transfer [O'neill *et al*, JACS '04]. However, very little is known at present about the underlying mechanisms. We use 2-aminopurine, a fluorescent base analog that can be included in DNA without disrupting the structure, to study dynamic motions and electronic processes in single bases. With ps fluorescence and absorption we characterize flipped conformations, transient opening and the formation of dark states by interaction with the neighboring bases [Somsen *et al* CPL '04]]. Our results provide a valuable tool to the study of mispair recognition, local melting, base flipping, protein binding and electron transfer.

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Structural analysis of curved DNA by atomic force microscopy

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Curvature of DNA plays an essential role in packing, transcription, regeneration and regulation of genomes. The combination of high-resolution atomic force microscopy and single-molecule force spectroscopy is a suitable tool for imaging individual DNA-fragments before and after the investigation of their mechanical properties. The objective of this study was to investigate the impact of DNA-topology, i.e. the supercoiled state, on the mechanical properties by means of force spectroscopy in conjunction with imaging. For this purpose curved DNA was adsorbed on mica under various conditions in order to determine the influence of salt concentration on the elasticity and appearance on the surface.

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Ligand-induced DNA condensation: choosing the model

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I test and compare different models for multivalent ligand-induced DNA condensation. Using ^{14}C-labelled spermidine^{3+}, I measure the binding to condensed DNA at micromolar to molar polyamine concentrations. DNA condenses and aggregates at a critical polyamine concentration. Spermidine^{3+} binding becomes highly cooperative at the onset of condensation. At higher concentrations the spermidine^{3+} binding to condensed DNA reaches a plateau with the degree of binding 0.7 (NH_4^+ / PO_3^-). Condensed DNA exists in a wide range of spermidine concentrations with the roughly constant degree of ligand binding. At even greater concentrations the degree of binding increases again. Further spermidine penetration between the double helices causes DNA resolubilization. I show that a simple two-state model without ligand-ligand interactions predicts qualitatively the reentrant aggregation behavior and the dependence on the ligand, Na^+ and DNA concentrations. However, the binding curves calculated in the frame of such models are inconsistent with the cooperative ligand binding to condensed DNA. Including the contact or long-range ligand-ligand interactions allows a better coincidence with the experiments. For example, a suitable McGhee-von Hippel contact cooperativity parameter is between 1.8 and 2.3, which is much smaller than the typical values for protein binding. Possible physical mechanisms for the observed cooperativity are discussed. This work was supported by INTAS (YSF 2002-141), BFFI (X04MC-031) and ISTC (À-301.2).

P-231

Chromatin compaction at the mono- and trinucleosomal level

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Gene activity and silencing are related to temporary and local changes in chromatin compaction. Factors influencing this include the ionic environment, presence of linker histones, post-translational modification of the core histones and DNA methylation. We studied the effect of some such factors on reconstituted mono- and trinucleosomes by measuring linker DNA distances in solution by FRET and by observing in SFM the conformation of trinucleosomes attached to a surface. Monovalent ions lead to approach of the linker DNA arms in all observed cases. Binding of linker histones induces further approach of the linker DNAs and decreases internucleosome distances. Selective acetylation of core histones causes differential effects on the DNA geometry. DNA methylation is not unequivocally associated with changes in the local compaction.

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Self-assembled layers of nucleic acid bases and nucleosides at the interfaces and the origin of life

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We have found that purine and pyrimidine derivatives currently occuring in nucleic acids posses an extraordinary high ability of self-association at the electrode surface and can form there by a two-dimensional (2-D) condensation a monomolecular layer (self-assembled monolayer – SAM), a compact film [1,2]. By this high condensation ability nucleic acid bases differ from most of the other purine and pyrimidine derivatives which currently do not occur in nucleic acids. This property was probably significant for the origin of life at the earth [3,4]. The effect of supporting electrolyte, pH value and substituents on the 2D condensation of nucleic acid bases and/or nucleosides will be shown.

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Posters
– From DNA to Chromatin –

P-233

Raman spectroscopic study of DNA modified by platinum cytostatics

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Platinum compounds constitute a new class of DNA-damaging agents. The first platinum anticancer drug introduced in clinic was cisplatin [(cis-diamminedichloroplatinum(II)]. This drug is highly effective in the treatment of testicular and ovarian cancer. There is a large body of experimental evidence that DNA is the critical target for the cytostatic activity of platinum compounds. Platinum complexes form several types of adducts, which occur in DNA with a different frequency and differently distort the conformation of DNA. Many biophysical and molecular-biological techniques has been used to describe such a distortions. Raman spectroscopy is powerful technique for examining both structural and thermodynamic properties of nucleic acids in solution.
The objective of the presented study was to confirm the sensitivity of Raman spectroscopy to structural changes induced in DNA by different classes of platinum complexes. This work presents the results obtained by using Raman spectroscopy for model system based on site-specific adducts formed by cis- and trans-DDP on synthetic oligonucleotides (monoaduct, 1,2-intra, 1,3-intra and interstrand crosslinks). We will also present the analysis of Raman spectra of DNA modified by first and second generation of antitumor active platinum complexes having cis and/or trans geometry of leaving ligands and novel bifunctional trinuclear platinum complexes.
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P-234

Dielectric spectroscopy of genomic DNA solutions

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Transport of electrical signals in complex biosystems, and in particular in the key molecule of DNA is of high interest. The anisotropic structure of DNA implies that the signaling process should be extremely sensitive to the local environment of the double helix. Revealing the dynamical properties of DNA as a function of its aqueous environment is certainly fundamental for macromolecular mechanisms in the cell.
Low frequency dielectric spectroscopy was employed, in the frequency range of 100 Hz to 100 MHz. This is a non-destructive tool, which can be used for detecting and quantifying the polarization response of DNA in aqueous environment. A wide concentration range of salmon sperm and calf-thymus Na-DNA solutions in pure water, and in NaCl electrolyte of varying ionic strength was investigated.
We discuss the possible assignments of observed relaxation modes taking into account the value and the concentration dependence of the dielectric relaxation parameters obtained from fits to the generalized Debye function. Persistence length is identified along with other characteristic length scales. We also discuss the influence of DNA on its environment, that is, on the effective diffusion constants of ions in solution.

P-235

Chromatin dynamics and structure studied at the single molecule level

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The DNA of all eukaryotes is arranged into a highly organized structure called chromatin whose fundamental unit is the nucleosome (NS). It is now established that chromatin is a dynamic structure that actively participates to gene regulation.
Single molecule methods have already proved powerful in providing important information about chromatin structure and dynamics (1,2). Here, we describe new and complementary tools to study real time chromatin dynamics with excellent temporal and spatial resolution.
We first investigate quantitatively chromatin assembly in real time with fluorescence microscopy (2,3) and show that it strongly depends on histones modifications and on their chaperones (4). Then, we examine the mechanical response of nucleosome arrays under tension and torsion with magnetic tweezers (5). We demonstrate that NSs can undergo a structural transition under torsion in order to release rotational strain. A model, confirmed by bulk and single molecule experiments, is proposed to account for this process. Finally, the biological relevance of these results is discussed.
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Posters

– Channels and Receptors –

P-236

Effects of divalent cations on the selectivity of the bacterial porin OmpF

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The OmpF channel is a porin found in the outer membrane of the bacteria *Escherichia Coli*. The main function of porins, which are commonly found among bacteria with similar functions, is to control the passage of small hydrophilic molecules through the membrane. They are also the pathway of antibiotics. The noticeable influence of divalent ions on the conductive properties of ion channels has been studied in electrophysiology for decades. Thus, it is known that an increase in the concentration of divalent ions leads to a shift in the conductance and a decrease in the reversal potential. We have addressed this issue in a series of experiments where OmpF channels are reconstituted in planar bilayers bathed in $MgCl_2$ and $CaCl_2$ solutions. The recorded data differ completely from those found in monovalent salts. Thus, under the same concentration gradient the reversal potential changes its polarity when switching from divalent cations to univalent cations. We suggest that this may be due to a combined effect of the charge screening and binding to some channel residues. Both factors are analyzed in the light of a PNP transport model for the ion permeation across the channel.

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Structural characterization of the i3 loop of the V2 Receptor and its complex with the P32 protein

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The V2 vasopressin receptor belongs to the widespread family of seven transmembrane receptors. Binding of vasopressin to the V2 receptor causes a structural rearrangment and subsequent binding to the Gαs protein leading to the production of cAMP. Defective V2 receptors are responsible for the congenital nephrogenic diabetes insipidus. The coupling of the V2 receptor with the Gαs protein is at least partly mediated by the third intracellular loop i3. This intracellular loop has a central importance since it is also thought to be implicated in the contact between protomers within the native dimer (Granier et al., 2004). Using a proteomic approach, we have previously shown that this loop may be also involved in additional interactions with other proteins, such as the P32 protein, an acidic ubiquitous protein (Granier et al., submitted). This opens the avenue for new mechanisms of signal transduction pathways for the V2 receptor and for GPCRs in general. Due to its central importance in signal transduction, we have undertaken the structural study of the i3 loop alone or in complex with protein partners. We present here the NMR structure of the i3 loop and the preliminary biophysical characterization of the P32/i3 complex.

Granier et al. (2004) J. Biol. Chem., 279, 50904-50914.

P-238

Fluorescence lifetime spectroscopy of KcsA reveals the existence of two gates in the permeation pore

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While it is generally agreed that the opening of the pore bundle crossing is a prerequisite for ion conduction, it still remains to be elucidated if the opening is also sufficient for pore conduction or if opening of a second gate near the selectivity filter is required. We addressed this problem by determining fluorescence lifetimes of KcsA as a function of pH labeled near C-terminus of TM2 right below the bundle crossing. The measurements were done with purified, labeled and reconstituted channels in lipid vesicles. The bound fluorophore showed two lifetimes, 3 ns and 1 ns, which were independent of pH. The relative amplitude of fast to slow lifetimes increased with lower pH. Since the pH dependence correlated well with the open probability of KcsA, we interpreted the two lifetimes as coming from two populations of channels either with closed (3 ns) or open (1 ns) bundle. The mutation A73E in the p-loop had a much higher open probability than wildtype KcsA, determined by Rb^+-flux and in bilayer experiments. A73E showed the same fraction of fast lifetimes, indicating that the open probability of the bundle crossing does not change in this mutant. These results suggest the existence of a second gate near the selectivity filter, serial to the bundle crossing, which is predominantly closed in wildtype KcsA, but kept open in the mutants with high open probability.

P-239

The complex response of neurons to nitric oxide: from channel to the whole-cell level

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Nitric oxide (NO) is known to be an inter-and intracellular messenger in the nerve cells. In the present work we show that NO influences various neuronal structures and causes prolonged interrelated changes of neurons' properties. By means of patch-clamp recording and fluorescent microscopy we studied effect of NO on the ion channel activity, amount of bound Ca^{2+} and mitochondria function. Using interference microscopy combined with data-series analysis we investigated NO influence on the neurons' refractive index (RI) and the dynamics of its changes. Our preparations were neurons of the pond snail and medical leech. As NO donor we used sodium nitroprusside. It was shown that exogenous NO firstly activates voltage-dependent K-channels and then evokes desorption of Ca^{2+} (Ca^{2+}_{mb}) from the surfaces of plasma and organelles membranes and Ca^{2+} exit from Ca^{2+}-binding proteins. Change of Ca^{2+}_{mb} amount alters membrane surface charge and can influence activity of the membrane proteins. NO causes the decrease in neurons' autofluorescence and long-lasting depolarisation of mitochondria, which can bring to the release of Ca^{2+} from their matrix. For the first time we showed that NO influences neurons' RI due to the cooperative processes evoked by NO in the plasma membrane and cytoplasm. We suggest that prolonged changes of organelles properties may be among mechanisms that provide signal transduction.

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– Channels and Receptors –

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Self-organized criticality in ion channel dynamics: the simple model

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The existing evidence of multifractality and long-term memory in the dwell times series of various ion channels contradicts with the traditional framework of modelling ion channel kinetics as Markov processes. Fractal properties of ion channel dynamics can lead to complex patterns of activity in cells and need to be correctly modelled.
The sandpile-like model of self-organized criticality (SOC) is used to describe conformational dynamics in the unstructured cytoplasm subunits of a Maxi-K channel. The gating is coupled to the SOC parts as a threshold-driven switching between two discrete conformations of S_6 helixes in the membrane. If the mean energy of the SOC part exceeds a given threshold, the gates dwell in an open state, otherwise, the channel is closed. Changes in the membrane potential and temperature are introduced as lowering the threshold and the increase in energy increment in the system respectively. The dwell times series, produced by this system display long-term memory and multifractality that match those of real Maxi-K channel dwell times series (multifractal spectra and local Hurst exponents trains were analysed). These multifractal properties are robust and not likely to change with temperature and membrane potential. Randomizing of these series has lead to loss in both memory and multifractality.

P-241

Stability of hereditary spherocytosis mutants of the cytosolic domain of the erythrocyte AE1 protein

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Anion exchanger 1 (AE1, Band 3) is the predominant membrane protein of erythrocytes. Its C-terminal domain functions as a Cl^-/HCO_3^- exchanger while its N-terminal cytosolic domain (cdb3) anchors the cytoskeleton to the membrane. Hereditary spherocytosis (HS) is a hemolytic anemia characterized by fragile spherocytes due to weakened interactions between the membrane and the cytoskeleton. Three cdb3 mutations associated with HS have normal red cell levels of AE1 but decreased levels of cytoskeletal protein 4.2. Band 3 Memphis I (K56E), a cdb3 mutant not associated with HS, has a reduced mobility on SDS-PAGE. Three HS cdb3 mutants (E40K, G130R and P327R) and the K56E mutant were expressed in *E.coli*. Sedimentation experiments showed that the purified proteins are dimers. Circular dichroism (CD) analysis showed no changes in secondary structure. Thermal denaturation monitored by CD and calorimetry and urea denaturation monitored by fluorescence revealed the following order of stability: K56E >WT >G130R >E40K >P327R. Tryptic digests showed that residue 40 was exposed, while residue 56 was not. The results show that HS mutations slightly destabilize the cdb3 protein and K56E is a stabilizing mutation. This destabilization may affect protein 4.2 binding resulting in its degradation. (Supported by CIHR and The Canadian Blood Services Graduate Fellowship).

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Role of the Ca2+ channel I-II loop in Voltage and Calcium dependent inactivation

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Calcium influx via voltage-gated Ca channels is a key step in initiating various vital cellular functions. Toxic Ca^{2+} overload is prevented by channel inactivation occurring through two different processes identified by their primary trigger: voltage or intracellular Ca^{2+}. In physiological situations, cardiac L-type ($Ca_V1.2$) Ca^{2+} channels inactivate primarily via Ca^{2+}-dependent inactivation (CDI), while neuronal P/Q ($Ca_V2.1$) Ca^{2+} channels use preferentially voltage-dependent inactivation (VDI). In certain situations however, these both types of channels have been shown to be able to inactivate by both processes.
From a structural view-point, the rearrangement occurring during CDI and VDI is not precisely known, but functional studies have underlined the role played by at least 2 channel sequences: a C-terminal binding-site for the Ca^{2+} sensor calmodulin, essential for CDI, and the loop connecting domains I and II, essential for VDI. The conserved regulation of VDI and CDI by the auxiliary channel β subunit strongly suggests that these two mechanisms may use a set of common protein-protein interactions that are influenced by the auxiliary β subunit. We will present new data on L-P/Q ($Ca_V1.2$/$Ca_V2.1$) chimeric and mutated channels obtained in Ba^{2+} and Ca^{2+} using different set of auxiliary subunits to analyse the role of the I-II loop and the channel C-terminus in VDI and CDI. These data reveal some of the essential steps in Ca^{2+} channel inactivation. Supported by CNRS, INSERM, AFM, ARC, and FRC.

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Structures of iron-siderophore outer membrane receptors from P. aeruginosa

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Under iron-limited conditions, the pyoverdine (Pvd) and pyochelin (Pch) outer membrane receptors, FpvA and FptA respectively, from the human pathogen *P. aeruginosa*, translocate the ferric-Pvd and ferric-Pch across the outer membrane involving the inner membrane energy transducing complex TonB-ExbB-ExbD. We solved the structures of FpvA loaded with iron-free Pvd at 3.6 Å resolution, Pvd-Fe at 2.8 Å and FptA bound to Pch-Fe at 2.0 Å resolution. Both receptors are folded in two domains: a transmembrane 22-stranded β-barrel domain occluded by the N-terminal domain. Pch provides a tetradentate coordination of iron and Pvd an hexadentate coordination. On the basis of biochemical studies and according to these structural studies two different mechanisms are involved in the iron-siderophore binding onto its receptor. The TonB box, involved in intermolecular contacts with TonB, is observed in an extended conformation in FpvA bound to Pvd while it is disordered in FpvA bound to the Pvd-Fe and FptA. Comparison of these first structures from a bacterium other than *E. coli* with the known structures of the *E. coli* siderophore transporters reveals a high structural homology. The N-terminal part containing the TonB box and the orientation of extracellular loops involved in the closure of the ligand binding site differ between the structures according to the siderophore status. All these results suggest a similar allosteric mechanism of the iron-siderophore transport across the membrane.