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Abstracts

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Plenary talks

O-001
View into the ribosomal exit tunnel
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Ribosomes, the universal polymerases for the translation of the genetic code into proteins, possess an elongated tunnel through which the nascent protein progress until they emerge into the cell. While traveling through the tunnel the nascent proteins can interact with specific tunnel wall components capable of imposing transient or a relatively long elongation arrest. Furthermore, internal architecture and flexibility allow response to cellular signals, thereby provides a mechanism for gene regulation.

O-002
The interplay between actin dynamics and membrane tension determines the shape of moving cells
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A central challenge in cell motility research is to quantitatively understand how numerous molecular building blocks self-organize to achieve coherent shape and movement on cellular scales. We focus on one of the classic examples of such self-organization, namely lamellipodial motility, in which forward translocation is driven by a treadmilling actin network. We combine detailed measurements of lamellipodial morphology, spatio-temporal actin dynamics and membrane tension, with mathematical modelling to explain how global shape and speed of the lamellipodium emerge from the underlying assembly and disassembly dynamics of the actin network within an inextensible membrane bag.

O-003
Structure determination of dynamic macromolecular complexes by single particle cryo-EM
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Macromolecular complexes are at the heart of central regulatory processes of the cell including translation, transcription, splicing, RNA processing, silencing, cell cycle regulation and repair of genes. Detailed understanding of such processes at a molecular level requires structural insights into large macromolecular assemblies consisting of many components such as proteins, RNA and DNA. Single-particle electron cryomicroscopy is a powerful method for three-dimensional structure determination of macromolecular assemblies involved in these essential cellular processes. It is very often the only available technique to determine the 3D structure because of the challenges in purification of complexes in the amounts and quality required for X-ray crystallographic studies.
In recent years it was shown in a number of publications that it is possible to obtain near-atomic resolution structures of large and rigid macromolecules such as icosahedral viruses. Due to a number of methodological advances there are now also great perspectives for high-resolution single particle cryo-EM studies of large and dynamic macromolecules. Successful high-resolution structure determination of dynamic complexes requires new biochemical purification strategies and protocols as well as state of the art electron microscopes and high-performance computing. In the future cryo-EM will thus be able to provide structures at near-atomic

resolution and information about the dynamic behavior of macromolecules simultaneously.

O-004
Detection and rapid manipulation of phosphoinositides with engineered molecular tools
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Polyphosphoinositides (PPIs) are ubiquitous lipid regulators of a variety of cellular processes serving as docking sites and conformational switches for a large number of signaling proteins. The localization and dynamic changes in PPIs in live cells have been followed with the use of protein domain GFP chimeras. In this presentation we will show experimental systems that allow rapid manipulation of the levels of PPIs in specific membrane compartments. We are also actively pursuing strategies that will allow us to map the distribution and possible functional diversity of the phosphatidylinositol (PtdIns) pools within intact cells since they are the precursors of PPIs. We will show our most recent progress in addressing this question: the use of a PtdIns specific PLC enzyme isolated from *Listeria monocytogenes* together with a highly sensitive diacylglycerol sensor to determine the distribution and also to alter the level of PtdIns in living cells. These studies reveal that a significant metabolically highly active PtdIns pool exists associated with tiny mobile structures within the cytoplasm in addition to the known ER and PM PtdIns pools. We will show our most recent data on the consequences of PtdIns depletion within the various PtdIns pools on PPI production and on the morphology and functions of various organelles.

O-005
Spectroscopic explorations of the nature of protein dynamics
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The functionality of proteins is known to be intimately related to the motion of their constituents on the atomic/molecular level. The study of microscopic motion in complex matter is often reduced to the observation of some average mean square atomic displacement, a first, very partial characterization of the dynamics. The marked crossover in the temperature dependence of such quantities in hydrated proteins around 200 K, the so called “dynamic transition” has been originally observed a quarter of century ago. The origin, nature and the key characteristics of the atomic motions behind this remarkable evolution of the mean square displacement in proteins remained controversial over the past decades. Recent analysis of Mössbauer, dielectric relaxation and neutron scattering spectroscopic data provide unambiguous evidence that this phenomenon is caused by the temperature dependence of a relaxation process spread over several orders of magnitude in the time domain, similarly to the β relaxation process observed in glasses. The review and critical analysis of the available data highlights the inherent ambiguities of commonly used data fitting approaches. Emerging evidence from model independent observations tend to exclude some of the proposed mechanisms.

O-006
Microbial Rhodopsins: light-gated ion channels and pumps as optogenetic tools in neuro- and cell biology
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Microbial Rhodopsins are widely used in these days as optogenetic tools in neuro and cell biology. We were able to show that rhodopsins from the unicellar alga *Chlamydomonas reinhardtii* with the 7 transmembrane helix motif act as light-gated ion channels, which we named channelrhodopsins(ChR1,2). Together with the light driven Cl^- pump Halorhodopsin ChR2 is used for the non-invasive manipulation of excitable cells and living animals by light with high temporal resolution and more important with extremely high spatial resolution The functional and structural description of this new class of ion channels is given (electrophysiology, noise analysis,flash photolysis and 2D crystallography). New tools with increased spatial resolution and extremely enhanced light sensitivity in neurons are presented. A perspective for basic neurobiology and for medical applications is given.

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O-007
The spatial organization of growth factor signaling systems in cells
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Our main objective is to elucidate how intracellular networks of nanometer-sized protein molecules process extracellular information thereby determining cellular phenotype that manifests on the micrometer scale. The cellular response to

extracellular signals consists of the induction of specific gene expression patterns and the re- organization in space and time of stereo-specific macromolecular interactions that endow the cell with its specific morphology. We develop quantitative experimental and computational approaches to derive and conceptualize physical principles that underlie these dynamics of signal processing and cellular organization. We have an experimental emphasis on functional microscopic imaging approaches at multiple resolutions to study the localization and dynamics of protein reactions/interactions, maintaining the inherent spatial organization of the cell. We have a strong recursion between computation of molecular dynamics in realistic cell geometries as sampled by microscopy, and experiments that reveal the dynamic properties of networks in living cells. We investigate the cellular topography of activities that transmit signals from receptors at the cell surface. Here we ask, how spatial partitioning of intracellular signalling activities is achieved by the causality structure of the signalling network, and how this partitioning affects signal response. This entails the experimental elucidation of connections between reactions and the determination of enzyme kinetic parameters in living cells.

O-008

Molecular photovoltaics mimic photosynthesis

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The field of photovoltaic cells has been dominated so far by solid state p-n junction devices made e.g. of crystalline or amorphous silicon, profiting from the experience and material availability of the semiconductor industry. However, there is an increasing awareness of the possible advantages of devices referred to as “bulk” junctions due to their interconnected three-dimensional structure. Their embodiment departs completely from the conventional flat p-n junction solid-state cells, replacing them by interpenetrating networks. This lecture focuses on dye sensitized mesoscopic solar cells (DSCs), which have been developed in our laboratory. Imitating natural photosynthesis, this cell is the only photovoltaic device that uses a molecular chromophore to generate electric charges from sunlight and that accomplishes the separation of the optical absorption from the charge separation and carrier transport processes. It does so by associating the molecular dye with a film constituted of tiny particles of the white pigment titanium dioxide. The DSC has made phenomenal progress, present conversion efficiencies being over 12 percent for single junction and 16 percent for tandem cells, rendering the DSC a credible alternative to conventional p-n junction devices. Molecularly engineered porphyrine have recently even reached over 13 percent efficiency when used in conjunction with a new redox electrolyte. Commercial large scale production of flexible DSC modules has started in 2009. These solar cells have become viable contenders for large-scale future solar energy conversion systems on the bases of cost, efficiency, stability and availability as well as environmental compatibility.

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O-009

Organizing principle of the plasma membrane: three-tiered meso-scale domain architecture revealed by single-molecule tracking

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Single-molecule imaging and tracking techniques that are applicable to living cells are revolutionizing our understanding of the plasma membrane dynamics, structure, and signal transduction functions. The plasma membrane is considered the quasi-2D non-ideal fluid that is associated with the actin-based membrane-skeleton meshwork, and its functions are likely made possible by the mechanisms based on such a unique dynamic structure, which I call membrane mechanisms. My group is largely responsible for advancing high-speed single molecule tracking, and based on the observations made by this approach, I propose a hierarchical architecture of three-tiered meso-scale (2-300 nm) domains as fundamental organizing principles of the plasma membrane. The three tiers I propose are the following.
[Tier 1] 30-200 nm compartments made by partitioning the entire plasma membrane by the membrane-associated actin-based meshwork (membrane skeleton: fences) and its associated transmembrane proteins (pickets). Since the entire plasma membrane is partitioned by these structures, and the membrane skeleton provides important platforms for the molecular interactions and pools, membrane compartments are the most basic tier for the plasma membrane organization.
[Tier 2] Meta-stable 1-5 nm raft domains that can be turned into stable $\sim$10-20-nm domains (receptor-cluster rafts), based on ligand-induced homo-dimers of glycosylphosphatidylinositol (GPI)-anchored receptors (coupling with [Tier 3]) and facilitated by raft-lipid interactions.
[Tier 3] Protein complexes of various sizes (3-10 nm) and lifetimes.
I will also talk about how domains of Tiers 2 and 3 are coupled to the membrane partitioning (Tier 1). The concept of the three-tiered domain architecture of the plasma membrane and the cooperative interactions of different tiers provides a good perspective for understanding the mechanisms for signal transduction and many other functions of the plasma membrane.

Biomolecular interactions

P-010

Effects of electromagnetic fields on interaction between Norfloxacin and Human Serum Albumin

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Keywords: Human serum albumin, Norfloxacin, fluorescence, electromagnetic fields

Introduction: In the present study we investigate the effects of electromagnetic fields (EMF) on the binding of Norfloxacin (NRF) to Human Serum Albumin (HSA) by fluorescence, three-dimensional fluorescence and uv-visible spectroscopic approaches. HSA is the most abundant protein in human blood plasma which works as a carrier that transports different materials in the body. NRF is used to treat variety of bacterial infections. It works by stopping the bacterial growth.
Methods: HSA, NRF and potassium phosphate buffer were purchased from sigma. Fluorescence Spectrofluorometer, UV-vis spectrophotometer, Three-dimensional fluorescence and a home-built EMF generator apparatuses were used.
Results: Results obtained from this study indicated that NRF has a strong ability to quench HSA in 280 nm. In addition, there was a slight blue shift, which suggested that the microenvironment of protein became more hydrophobic after addition of NRF. Moreover, synchronous fluorescence demonstrated that the microenvironment around Tyrosine (Tyr) had a trivial increase. These, and the results of HSA–NRF in the presence of EMF with 1 KHz, illustrates the same results inferred from quenching and blue shift. However, there was a significant decrease in K_{SV} of NRF with HSA in presence of EMF exposure. Moreover, the binding parameters including the number of binding sites and the binding constant were calculated form Hill equation.
Conclusion: It was shown that NRF could induce conformational changes in HSA both in the absence and presence of EMF with no significant difference. Yet, the affinity is decrease significantly in the presence of EMF. The clinical implications are discussed in detail.

P-012

Characterization of the biochemical properties and biological function of the formin homology domains of drosophila daam

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We characterised the properties of Drosophila melanogaster DAAM-FH2 and DAAM-FH1-FH2 fragments and their interactions with actin and profilin by using various biophysical methods and in vivo experiments. The results show that while the DAAM-FH2 fragment does not have any conspicuous effect on actin assembly in vivo, in cells expressing the DAAM-FH1-FH2 fragment a profilindependent increase in the formation of actin structures is observed. The trachea specific expression of DAAM-FH1-FH2 also induces phenotypic effects leading to the collapse of the tracheal tube and lethality in the larval stages.
In vitro, both DAAM fragments catalyze actin nucleation but severely decrease both the elongation and depolymerisation rate of the filaments. Profilin acts as a molecular switch in DAAM function. DAAM-FH1-FH2, remaining bound to barbed ends drives processive assembly of profilin-actin, while DAAM-FH2 forms an abortive complex with barbed ends that does not support profilinactin assembly. Both DAAM fragments also bind to the sides of the actin filaments and induce actin bundling. These observations show that the Drosophila melanogaster DAAM formin represents an extreme class of barbed end regulators gated by profilin.

P-013

Electron spin echo studies of free chain-labelled stearic acids interacting with β-lactoglobulin

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β-lactoglobulin (βLG) binds non-covalently fatty acids within its central calyx, a cavity in the barrel formed by the strands βA- βH.
We present results of pulsed Electron Paramagnetic Resonance (EPR) spectroscopy on the interaction of βLG with stearic acids spin-labelled at selected positions, *n*, along the acyl chain (*n*-SASL, $n = 5,7,10,12,16$).
D_2O-Electron Spin Echo Envelope Modulation (ESEEM) Fourier transform spectra indicate that all segments of the bound chains in the protein binding site are accessible to the solvent. The extent of water penetration decreases progressively on moving from the first segments toward the terminal methyl end of the chain. About 50% of the nitroxides in the upper part of the chain ($n = 5,7$) are H-bonded by a single water molecule and this fraction reduces to 30% at the chain terminus ($n = 12,16$). A lower fraction of the nitroxides are H-bonded by two water molecules, and it decreases from about 15% to a vanishingly small value on going down the chain.
Echo-detected ED-EPR spectra reveal subnanosecond librational motion of small amplitude for both 5- and 16-SASL in the protein cavity. The temperature dependence of the librations is more marked for 16-SASL and it arises mainly from an increase in librational amplitude with increasing temperature.

P-014

Interaction of two putative fusion peptides from SARS-CoV glycoprotein with model membranes

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Fusion peptides (FP) pertaining to the spike glycoprotein from severe acute respiratory syndrome (SARS) coronavirus are essential for the fusion between viral and host cellular membranes. Here we report a biophysical characterization of the interaction of two putative FPs with model membranes. Fluorescence and DSC experiments showed that both peptides bind stronger to anionic than to zwitterionic lipid membranes. ESR spectra showed that TOAC-$SARS_{IFP}$ rotational dynamics is modulated by lipid composition and pH as compared to the spectrum of this peptide in solution. However, stearic acid spin labels reported no changes on the dynamic structure of zwitterionic micelles, whereas the whole chain of anionic surfactants was perturbed by the peptides. Finally, CD data revealed a predominant β-strand structure for $SARS_{FP}$ and an α-helix for $SARS_{IFP}$ in the presence of micelles, in contrast to their disordered structures in buffer. Overall the results point out that electrostatic and hydrophobic interactions are both important to the energetic behavior of peptide membrane interaction. These findings might provide a useful rationale for the elucidation of one of the steps involved in the fusion process, and thus help understanding the more general way of action of FPs at a molecular level.

P-015

Interaction of filamentous actin and ezrin within surface modified cylindrical nanopores

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Ezrin is a member of the ezrin-radixin-moesin (ERM) protein family that acts as a dynamic linker between the plasma membrane and the actin cytoskeleton and is hence involved in membrane organization, determination of shape and surface structures and other cellular processes. The protein is highly enriched in microvilli of polarized epithelial cells, where it binds filamentous actin (F-actin) with its C-terminal domain, while the N-terminal domain is connected to the plasma membrane via specific binding to L-α-phosphatidylinositol-4,5-bisphosphate (PIP_2).

Nanoporous anodic aluminum oxide (AAO) films provide similar dimensions as microvilli and are thus a versatile template to investigate the interaction of ezrin with F-actin within spationally confined areas. Owing to their optical transparency, functionalized AAOs can be used to measure the binding process of ezrin to a PIP_2 containing solid supported membrane by means of time resolved optical waveguide spectroscopy (OWS).

Confocal laser scanning microscopy (CLSM) will elucidate, whether F-actin binding to ezrin takes place within or atop the nanopores. Furthermore, elasticity mapping of F-actin filaments by means of atomic force microscopy will allow determining binding forces and the lateral tension of the actin cytoskeleton.

P-017

In vitro application of porphyrin photosensitisers on MCF7, HeLa and G361 tumour cell lines

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Tumour treatment presents a challenge to all scientists and clinicians. Contemporary methods like radiotherapy, chemotherapy or surgery have many undesirable side effects. Photodynamic therapy (PDT) seems to be one of alternatives which can be helpful in malignant cell therapy. PDT is not only limited to cancer treatment but is also used as an alternative for cardiovascular, skin and eye disease treatment. PDT employs photosensitive agents which need to be activated by light which is not harmful to a patient. The activated photosensitive agent provokes a formation of reactive oxygen species leading to cell damage or death. The phototoxicity of the two porphyrin photosensitizer (TMPyP, $ZnTPPS_4.12H_2O$) on the malignant cell lines (G361, HeLa, MCF7) irradiated with the 1 Jcm^{-2} doses was evaluated by ROS production assay, MTT assay and comet assay. Our results indicate higher efficiency of TMPyP over $ZnTPPS_4.12H_2O$. As for the photodynamic effectiveness of the used photosensitizers on chosen cell lines we found that HeLa cell line is the most sensitive to phototoxic damage induced by TMPyP.

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P-018

nmR analysis of the Respiratory syncytial virus M2-1 protein structure and of its interaction with some of its targets

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The respiratory syncytial virus (RSV) is a major cause of acute respiratory tract infections (bronchiolitis, pneumonia) in human and a leading cause of viral death in infants and immunocompromised patients. RSV genome is formed of a single non-segmented negative strand RNA which transcription and replication is ensured by a specific RNA-dependent RNA polymerase complex formed of the large (L) polymerase

subunit and of several cofactors. This complex has no cellular counterpart and represents an ideal target for antiviral drugs. Among the cofactors, M2-1 acts as an antitermination factor and increases the polymerase processivity. Its central domains has been shown, in vitro, to bind the phosphoprotein P and genomic RNA in a competitive manner. Here we report the nmR structure of this central domain and its interaction with P and RNA fragments. M2-1 shares structural similarity with VP30, a transcription factor of Ebola virus. The binding surfaces for RNA and P are distinct but overlapping. RNA binds to a basic cluster located next to residues found to be critical for transcription both in vitro and *in vivo* by mutational analysis. We speculate that M2-1 might be recruited by P to the transcription complex, where interaction with RNA takes place, stabilized by additional elements.

P-019
Force spectroscopy at the membrane-cytoskeleton interface: interactions between ezrin and filamentous actin

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Ezrin, a member of the ERM (ezrin/radixin/moesin) protein family, provides a regulated linkage between the plasma membrane and the actin cytoskeleton. It contributes to the organization of structurally and functionally distinct cortical domains participating in adhesion, motility and fundamental developmental processes. Ezrin is negatively regulated by an intramolecular interaction of the terminal domains that masks the F-actin binding site. A known pathway for activation involves the interaction of ezrin with phosphatidylinositol 4,5-bisphosphate (PIP_2) in the membrane, followed by phosphorylation of the threonine 567 residue in the C-terminal domain. To date, it is unclear to what extent both regulatory inputs contribute to the activation.
We developed an in vitro system that facilitates the specific analysis of the interaction forces between ezrin and F-actin by means of atomic force spectroscopy (AFM). Applying ezrin wild type and the pseudophosphorylated mutant protein ezrin T567D, respectively, permits to monitor the individual influence of phosphorylation on the F-actin-ezrin interaction. Thus, a thorough characterization of the acting forces at the ezrin-actin interface will elucidate the activation mechanism of ezrin.

P-020
PEG-ylated LDL particles: a potential drug delivery system

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Low-density lipoproteins (LDL) have proven to be useful vehicles for lipophilic drugs to cancer cells. To make this delivery system even more efficient, we have constructed nano-carrier by coating of LDL by polyethylene glycol (PEG). The hydrophilicity of PEG should reduce the interaction of LDL with other serum proteins and consequently decrease the redistribution of loaded drug from LDL to the (lipo)proteins. Dynamic light scattering was used for determination of hydrodynamic radius of LDL-PEG particles. CD spectroscopy measurements didn't reveal structural changes of apoliprotein B-100 (ligand for LDL receptors on cell surface), after conjugation of PEG with LDL. Interaction of LDL-PEG complexes with hypericin (Hyp) a natural photosensitizer was studied by fluorescence spectroscopy. We have demonstrated accumulation of higher number of Hyp in LDL-PEG than LDL particles. However, the kinetics of Hyp redistribution from Hyp/LDL-PEG complex to free LDL have similar parameters as those for the kinetics of Hyp transfer between non-modified LDL molecules. We suggest that Hyp molecules are mostly localized in the vicinity of the surface of the LDL-PEG particles and they are prone to redistribution to other serum proteins.

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P-021
Reactivity of aminophospholipid's polar head with glucose and arabinose

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Modification of the head-group of aminophospholipids by glycation and subsequent lipid oxidation affect membrane's structure causing cell death.[1] These processes are involved in the pathogenesis of aging[2] and diabetes.[3] Non-enzymatic glycation forms in the first step a Schiff base (SB), which rearranges to a more stable ketoamine, Amadori product, which leads to the formation of a heteregenous group of compounds (AGEs). Although several studies have been focused on identification of aminophospholipid glycation products,[4] less attention has been paid to kinetic mechanism of the reaction. For that reason, in the present work, we compare the kinetic reactivity of polar head-group of phosphatidylethanolamine (PE) and phosphatidylserine (PS), the two target phospholipids components of mammalian cell membranes. The reaction of PE and PS's head-group with glycating compounds (glucose and arabinose) was studied in physiological conditions by using nmR spectroscopy. The obtained formation rate constants for SB are lower than those determined for the SB of the peptide Ac-Phe-Lys with the same carbohydrates.[5] It suggests that the phosphate group may delay the glycation process. Moreover, the PS's head-group has a carboxilic group in the structure, which affects the stability of the SB.

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P-022

Ultrasensitive protein detection in nano-immuno assays based on DNA directed immobilization and atomic force microscopy nanografting

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We developed ultrasensitive, ELISA-like nanoimmuno assays suitable for proteomics/interactomics studies in low sample volumes. We exploit the approach of DNA microarray technologies applied to proteomics [1], in combination with atomic force microscopy (AFM) to generate functional protein nanoarrays: semisynthetic DNA-protein conjugates are immobilized by bioaffinity within a nanoarray of complementary ssDNA oligomers produced by AFM nanografting (NG). A nanoarray of different antibodies or synthetic molecular binders can be generated in a single operation, once the DNA nanoarray is produced. Moreover, NG allows adjusting the packing density of immobilized biomolecules to achieve optimum bio-recognition.
AFM-based immunoassays with these nanoarrays were shown to achieve detection limit of hundreds of femto Molar, in few nanoliters volumes, with very high selectivity and specificity [2]. To detect the hybridization efficiency of our devices, we run a combined experimental-computational study that provides quantitative relations for recovering the surface probe density from the mechanical response (AFMcompressibility measurements) of the sample.

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P-023

Distinct ubiquitin binding modes exhibited by the SH3 domains of CD2AP

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SH3 domains form a highly conserved family of protein domains. Recently, they were identified as a new class of ubiquitin binding domains. Several mechanistic differences have been proposed for ubiquitin binding to SH3 domains involved in the immune signaling pathway. A key affinity and specificity determinant has been appointed to residue Phe409 in Sla1 SH3-3 domain. We previously showed that the third SH3 domain (SH3-C) of CD2 associated protein (CD2AP), which possesses that key phenylalanine residue (Phe324), indeed binds to ubiquitin but in a different orientation to that in Sla1 SH3-3. In this work, we have performed structural and mutational analysis of the three SH3 domains of CD2AP and the third SH3 domain of CIN85. We have found that the first and second SH3 domains of CD2AP bind ubiquitin in a similar orientation to that in Sla1 SH3-3, as opposed to the third one, despite the high sequence homology the three CD2AP-SH3 domains share. We have also shown that SH3 domains displaying this different ubiquitin binding mode interact with higher affinity to ubiquitin molecules with extended C-terminus. We conclude that the CD2AP SH3-C interaction with ubiquitin constitutes a new ubiquitin binding mode involved in a different cellular function, thus changing the previously established mechanism of EGF-dependent CD2AP/CIN85 monoubiquitination.

P-024

Virtual screening for the discovery of authentic inhibitors against cytosolic 5'-nucleotidase II: therapeutic use and benefit in cancer treatment

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Nucleoside analogues used as anticancerous drugs can be rapidly degraded within treated cells, constituting a major obstacle of their therapeutic efficiency. Among the enzymes responsible for this degradation, the cytosolic 5'-nucleotidase II (cN-II) catalyses the hydrolysis of some nucleoside monophosphates. In order to improve the efficacy of anticancerous drugs and to define the precise role of cN-II, new original inhibitors have been developed against cN-II. Virtual screening of chemical libraries on the crystal structure has allowed us to identify very promising candidates that turned to be competitive inhibitors of cN-II. One molecule was included in the anticancerous treatment of tumoral cell lines in order to evaluate the potential benefit and could induce in fine a sensitization of certain anticancerous drugs. We also explore other inhibitors targeting the allosteric sites of this enzyme using a strategy that takes into account the dynamics of cN-II. The chemical structures of the newly identified allosteric inhibitors as well as the atomic interactions with enzyme residues will be presented. The final goal of this study is to find

molecules that can freeze the enzyme in a conformation for which its dynamics is severely limited and therefore its function.

O-025
Native mass spectrometry to Decipher interactions between biomolecules
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Mass spectrometry is generally understood as "molecular mass spectrometry" with multiple applications in biology (protein identification using proteomic approaches, recombinant protein and monoclonal antibody characterization). An original and unexpected application of mass spectrometry emerged some twenty years ago: the detection and the characterization of intact biological noncovalent complexes. With recent instrumental improvements, this approach, called native MS, is now fully integrated in structural biology programs as a complementary technique to more classical biophysical approaches (NMR, crystallography, calorimetry, SPR, fluorescence, etc.).
Native MS provides high content information for multiprotein complexes characterization, including the determination of the binding stoichiometries or oligomerization states, site-specificities and relative affinities. Recent developments of ion mobility / mass spectrometry instruments (IM-MS) provide a new additional level for MS-based structural characterization of biomolecular assemblies allowing size and shape information to be obtained through collisional cross section measurements.
These different aspects of native MS for structural characterization of biomolecular assemblies will be illustrated through several examples, ranging from multiprotein-complexes to protein/nucleic acid assemblies.

P-026
Atomic Force Microscopy in study of gum-arabic/ chitosan complexes
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Complex coacervation is a process which may result by electrostatic interaction between charged polysaccharides. It depends essential on pH, ionic strength and biopolymers properties like ratio, concentration and charge density.
In this case, the main work was to study the structural properties of a colloidal system of opposite charge – chitosan and gum-arabic by atomic force microscopy (AFM).
According to some of complexes show tendency to agglomerate. This depends on the molar ration of the macromolecules and their relative molecular weights.
AFM micrographs show, too, that some formation of irregular aggregates by both polymers were due to presence of non-charged polar monomers in chitosan molecule.
At higher gum-arabic/chitosan ratios biopolymer concentrations, coacervates appear like a core-shell miccelar structure composed of hydrophobic core (charge neutralized segments) stabilized by the excess component (positive zeta potential) and non-charged segments of gum arabic.

P-027
Interaction of human serum albumin with rutin theoretical and experimental approaches
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Human serum albumin (HSA) is the principal extracellular protein with a high concentration in blood plasma and carrier for many drugs to different molecular targets. Flavonoids are a large class of naturally occurring polyphenols widely distributed in plants. Rutin (quercetin-3-rutinoside) is the glycoside between flavonoids quercetin and disaccharide rutinose. Like other flavonoids, rutin displays anti-inflammatory and anti-oxidant properties. The interaction between HSA and rutin was investigated by fluorescence spectroscopy, *ab initio* and molecular modeling calculations.
Fluorescence titration was performed by keeping the HSA concentration (4 μM) constant and stoichiometrically varying the rutin concentration (1−4 μM). The emission spectra were obtained in the range of 305 to 500nm, with the excitation wavelength at 295nm. The obtained fluorescence data were corrected for background fluorescence and for inner filter effects. The Stern-Volmer quenching constant values were 3.722×10^5 and 1.868×10^5 M^{-1} at 298 and 303 K, respectively. From the modified Stern-Volmer association constants 2.285×10^5 (at 298 K) and 2.081×10^5 M^{-1} (at 303 K) were calculated the thermodynamic parameters $\Delta H = 14.048$ KJ mol^{-1}, $\Delta G_{298K} = -30.557$ KJ mol^{-1} and $\Delta G_{303\ K} = -30.834$ KJ mol^{-1}, and $\Delta S = 55.4$ KJ mol^{-1} K^{-1}.
Fluorescence quenching method was used also to study the binding equilibria thus determining the number of binding sites 1.085 and 1.028, and binding constant 1.094×10^6 M^{-1} and 0.255×10^6 M^{-1} at 298 and 303 K, respectively. The efficient quenching of the Trp214 fluorescence by rutin indicates that the binding site for the flavonoid is situated within subdomain *IIA* of HSA. The distance $r = 2.397$ nm between the donor (HSA) and the acceptor (Rutin) was obtained according to fluorescence resonance energy transference (FRET). Wavelength shifts in synchronous fluorescence spectra showed the conformation of HSA molecules is changed in the presence of rutin.
The structure of rutin utilized in molecular modeling calculation was obtained by Gaussian 98 program. The optimization geometry of rutin was performed in its ground states by using *ab initio* DFT/B3LYP functional with 6-31G(d,p) basis set used in calculations. The molecular electrostatic potential (MEP) was calculated to provide the molecular charge

distribution of rutin. The gap energy value between the HOMO and LUMO of the rutin molecule was about 4.22 eV which indicates that rutin is classified as a reactive molecule. From molecular modeling calculation the interaction between HSA and rutin was investigated using the AutoDock program package. The three-dimensional coordinates of human serum albumin were obtained from the Protein Data Bank (entry PDB code 1AO6) and of rutin were obtained from output optimization geometry of DFT. The best energy ranked result shows that rutin is localized in the proximity of single tryptophan residue (Trp214) of HSA that is in agreement with the fluorescence quenching data analysis.

P-029

The effect of Toxofilin on the structure of monomeric actin

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Actin is one of the main components of the intracellular cytoskeleton. It plays an essential role in the cell motility, intracellular transport processes and cytokinesis as well.
Toxoplasma gondii is an intracellular parasite, which can utilise the actin cytoskeleton of the host cells for their own purposes. One of the expressed proteins of *T. gondii* is the 27 kDa-sized toxofilin. The long protein is a monomeric actin-binding protein involved in the host invasion.
In our work we studied the effect of the actin-binding site of toxofilin$_{69\text{-}196}$ on the G-actin. We determined the affinity of toxofilin to the actin monomer. *The flourescence of the actin bound* ε-ATP was quenched with acrylamide in the presence or absence of toxofilin. *In the presence of toxofilin the accessibility of the bound* ε-ATP decreased, which indicates that the nucleotide binding cleft is shifted to a more closed conformational state.
The results of the completed experiments can help us to understand in more details what kind of cytoskeletal changes can be caused in the host cell during the invasion of the host cells by intracellular parasites.

P-030

Porphyrin conjugates in virus inactivation—role of porphyrin binding and singlet oxygen production

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Here we tested the virus inactivation capability of tetra-peptide conjugates of meso-tri(4-*N*-methylpyridyl)-mono-(4-carboxyphenyl)porphyrin (TMPCP) or meso-5,10-bis(4-*N*-methylpyridyl)-15,20-di-(4-carboxyphenyl)porphyrin (BMPCP) in the dark and upon irradiation.
T7 bacteriophage, as a surrogate on non-enveloped viruses was selected as a test system. Both TMPCP and BMPCP and their peptide conjugates proved to be efficient photosensitizers of virus inactivation. The binding of porphyrin to phage DNA was not a prerequisite of phage photosensitization, moreover, photoinactivation was more efficiently induced by free than by DNA bound porphyrin.
Mechanism of photoreaction (Type I. versus Type II) and the correlation between DNA binding, singlet oxygen production and virus inactivation capacity was also analyzed.
DNA binding reduced the virus inactivation due to the reduced absorbance and singlet oxygen production of bound photosensitizer, and altered mechanism of photoinactivation. As optical melting studies of T7 nucleoprotein revealed, photoreactions of porphyrin derivatives affected the structural integrity of DNA and also of viral proteins, even if the porphyrin did not bind to NP or was selectively bound to DNA.

P-031

Antimicrobial photodynamic therapy of porphyrins on bacterial cells

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Keywords: Porphyrin, Photosensitizer, Cyclodextrin, Bacterial Strains

Photodynamic therapy is usually used against malignant and non-malignant tumors. Nowadays due to resistance of bacterial strains we look for a new antimicrobial strategy to destroy bacteria with minimal invasive consequences. Development antimicrobial technology combines nontoxic photosensitizer, visible light of appropriate wavelength and generation reactive oxygen species. In this work photosensitizers TMPyP and ZnTPPS4 are investigated for photodynamic antimicrobial therapy. We tested these two photosensitizers on two bacterial strains for comparison: Gram – positive *S. aureus* and Gram – negative *P. aeruginosa*. We applied photosensitizers alone and bound in the complex created with hp-β-cyclodextrin and we compared the effectiveness to bacteria. The light emitting diodes (LEDs 414 nm) were used as a source in PDT at the doses 0, 25, 50 and 100 J. Bacteria were grown in the presence of photosensitizers at the concentration from 0.78 to 100 μM. Up to now our result suggest that TMPyP and ZnTPPS4 are efficient alone and the efficiency even more increases in complex created with hp-β-cyclodextrin.
This work was supported by the grant projects 2192/2011/G3 from the Ministry of Education, Youth and Sports of the Czech Republic, 303/09/H048 from the Czech Science Foundation, CZ.1.05/2.1.00/01.0030 and LF_2011_009.

P-032

Characterization of tetracaine: cyclodextrin inclusion complexes by nuclear magnetic resonance

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Anesthesia is a medical milestone (Friedman & Friedland, Medicine's 10 Greatest Discoveries, 2000) and local anesthetics (LA) are the most important compounds used to control pain in surgical procedures. However, systemic toxicity is still a limitation for LA agents as well as low solubility, as for tetracaine (TTC). Approaches to improve LA effects include macrocyclic systems formation, such as in cyclodextrins (CD). We have studied complexes formed between TTC and β-CD or hydroxylpropyl (HP)-β-CD through nmR and other (UV-VIS, fluorescence, DSC and X-ray diffraction) techniques. At pH 7.4 a 1:1 stoichiometry of complexation was detected for both complexes, with association constants of 777 M^{-1} and 2243 M^{-1} for TTC:β-CD and TTC:HP-β-CD, respectively. The nuclear overhauser nmR data disclosed trough the space proximities between hydrogens H_h and H_i - at the aromatic ring of TTC - and hydrogens from the inner cavity of the cyclodextrins, allowing us to propose the topology of TTC:CD interaction. Complex formation did not curb TTC association with model (liposomes) and biological membranes since the total analgesic effect (infraorbital nerve blockade in rats) induced by 15mM TTC increased 36% upon complexation. Supported by (FAPESP # 06/121-9, 03838-1) Brazil.

P-033

ITC as a general thermodynamic and kinetic tool to study biomolecule interactions

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Isothermal Titration Calorimetry (ITC) is a powerful technique for thermodynamic investigations that is little used to obtain kinetic information. We have shown that, in fact, the shape of the titration curves obtained after each ligand injection is strictly governed by the kinetics of interaction of the two partners. A simple analysis allowed us to explain several facts (*e.g.* the variation of time needed to return to equilibrium during a titration experiment). All simplifications were further released to obtain a very realistic simulation of an ITC experiment. The method was first validated with the binding of the Nevirapine inhibitor onto the HIV-1 Reverse transcriptase by comparison with results obtained by Biacore™. Importantly, for more complex systems, the new method yields results that cannot be obtained in another way. For example, with the *E. coli* transcription-regulator Thiamine PyroPhosphate riboswitch, we could resolve **kinetically and thermodynamically** the two important successive steps: (1) the binding of the TPP ligand and (2) the subsequent RNA folding. Our results show that initial TPP binding is controlled thermodynamically by TPP concentration, whereas the overall transcription regulation resulting from RNA folding is kinetically controlled.

P-034

Characterization of human primary amine oxidase/Sialic acid binding Ig-like lectin 10 interaction

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Leukocyte trafficking into inflamed tissues from the blood vessels is essential in maintaining health. Human membrane primary amine oxidase (hAOC3) is involved in this trafficking. The mechanism of the role of hAOC3 in the trafficking is unknown, but its amine oxidase activity is important.
Recently the first hAOC3 counter molecule was identified being sialic acid binding Ig-like lectin -10 (Siglec-10; Kivi *et al.*, 2009, *Blood* **114**:5385-5392). Siglec-10 is an adhesion molecule expressed on the B-cells, monocytes and eosinophils. The hAOC3 binding of Siglec-10 site has been suggested to be in the second C2 domain of Sigec-10. For the characterization of hAOC3/Siglec-10 interaction we have cloned Siglec-10 fragments and produced the protein in insect cells. We have been able to demonstrate specific interaction between hAOC3 and Siglec-10 fragment. In addition, the fragment is processed by hAOC3. This is the first time that natural ligand of hAOC3 has been shown to be processed using purified proteins.

P-035

Investigating the oligomerization state of cyan fluorescent protein, from cell cytoplasm to solution

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The sensitivity of GFP-like fluorescent proteins to their environment is a potential source of artifacts in protein-protein interaction studies based on FRET. Besides parameters that vary in the course of metabolic processes, such as pH or reactive oxygen species concentration, protein expression level is likely to influence the fluorescence properties of

GFPs, due to their tendency to dimerize at high concentration. We have characterized for the first time the self-association properties of CFP (Cyan Fluorescent Protein), the fluorescent protein mostly used as FRET donor. We found that the fluorescence quenching observed at high expression level in the cell cytoplasm and the fluorescence depolarization measured at high concentration in vitro are insensitive to the A206K mutation, shown to dissociate other GFP dimers. Both phenomena are satisfactorily accounted for by a model of non-specific homo-FRET between CFP monomers due to molecular proximity. Modeling the expected contributions to fluorescence depolarization of rotational diffusion, homo-FRET within a hypothetical dimer and proximity homo-FRET shows that CFP has a homo-affinity at least 30 times lower than GFP. This difference is due to an intrinsic mutation of CFP (N146I), originally introduced to increase its brightness and that by chance also disrupts the dimers.

P-036

Coupling of the hydration shell of B-DNA to conformational substates and peptide recognition studied by time-resolved FTIR spectroscopy

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Biomolecular recognition typically proceeds in an aqueous environment, where hydration shells are a constitutive part of the interacting species. The coupling of hydration shell structure to conformation is particularly pronounced for DNA with its large surface to volume ratio. Conformational substates of the phosphodiester backbone in B-DNA contribute to DNA flexibility and are strongly dependent on hydration. We have studied by rapid scan FTIR spectroscopy the isothermal B_I-B_{II} transition on its intrinsic time scale of seconds. Correlation analysis of IR absorption changes induced by an incremental growth of the DNA hydration shell identifies water populations w_1 (PO_2^--bound) and w_2 (non-PO_2^--bound) exhibiting weaker and stronger H-bonds, respectively, than those in bulk water. The B_{II} substate is stabilized by w_2. The water H-bond imbalance of 3-4 kJ mol^{-1} is equalized at little enthalpic cost upon formation of a contiguous water network (at 12-14 H_2O molecules per DNA phosphate) of reduced Υ(OH) band width. In this state, hydration water cooperatively stabilizes the B_I conformer via the entropically favored replacement of w_2-DNA interactions by additional w_2-water contacts, rather than binding to B_I-specific hydration sites. Such water rearrangements contribute to the recognition of DNA by indolicidin, an antimicrobial 13-mer peptide from bovine neutrophils which, despite little intrinsic structure, preferentially binds to the B_I conformer in a water-mediated induced fit. In combination with CD-spectral titrations, the data indicate that in the absence of a bulk aqueous phase, as in molecular crowded environments, water relocation within the DNA hydration shell allows for entropic contributions similar to those assigned to water upon DNA ligand recognition in solution.

P-037

Segmental-labeling expression of SH3 domains of CD2AP protein to study interaction with their ligand

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Transient and low affinity interactions within the cell can be enhanced by the combination of more than one domain. Up to now most of the effort has been put on the study of the regulation in the affinity and specificity of the binding to isolated single domains but little is known about the effect of the presence of a second or third domain. Multiple examples of proteins containing tandem domains exist in the genome like the CIN85/CMS family of adaptor proteins. In this family all three N-terminal SH3 domains are involved in a wide variety of different interactions, they share higher similarity among themselves than to any other SH3 domains, suggesting an overlapping specificities in binding. CD2 associated protein (CD2AP) is an adaptor protein and belongs to this family, its N-terminus consists of three SH3 domains and the interaction of each one of them with its target(-s) might be ultimately modulated by the presence of its next-door-neighbor. In this work we present the expression and purification of the tandem CD2AP-SH3A/SH3B produced by segmental labeling techniques that allow us to express the domains with different isotopic label, improving the nmR signal and facilitating to study the interaction of the natural ligand in the presence of next-door-neighbor domain.

P-038

Kinetics of the interaction of DDP with DNA and nuclear proteins

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Platinum based coordination compounds are considered some of the most effective anti-tumor drugs. One of the most effective compounds in this group is cisplatin (*cis*-diamminedichloroplatinum(II) or cis-DDP). It is used in treatment of several types of tumors, yet its detailed mechanism of action remains unknown.

The anti-tumor mechanism of cis-DDP is based on its ability to form DNA crosslinks. It has been shown that sulfur containing amino acids are possible intermediates for DNA-cisplatin interactions. Proteins that contain such amino acids may be involved in transportation of cis-DDP into the cell nucleus. One of the most interesting examples of such a protein is non-histone protein HMGB1, which demonstrates an ability to interact with the DDP molecule. It has also been shown that HMGB- proteins preferentially bind to DNA platinum adducts.

Our research is focused on kinetic aspects of interactions of DDP with DNA and proteins in aqueous solutions. Using UV-spectroscopy, electrophoresis, kinetic and thermodynamic approaches we investigated DNA-DDP and HMGB-DDP interactions. Using chemometrics and principle component analysis we have determined kinetic constants of the major reactions.

P-039

Quantifying Molecular Partition of Supramolecular Complexes in Lipid Model Systems – Studies on Dengue Virus Supramolecular Components

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There are plenty of molecules that exert their effects at the cell membrane. The evaluation of these interactions, frequently quantified by the Nernst lipid/water partition constant (Kp), helps to elucidate the molecular basis of these processes. We present here a recently derived and tested method to determine Kp for single solute partitions using ζ-potential measurements. The concept was then extended to the interaction of supramolecular complexes with model membranes. A simultaneous double partition with an aqueous equilibrium is considered in this partition model. The results were validated by dynamic light scattering - DLS, ζ-Potential, fluorescence spectroscopy and laser confocal microscopy experiments. We evaluated the interaction of supramolecular complexes (peptides derived from dengue virus proteins with oligonucleotides) with LUV to study our biophysical models. Dengue virus (DV) infects over 50-100 million people every year and may cause viral hemorrhagic fever. No effective treatment is available and several aspects of its cellular infection mechanism remain unclear. The extension of the interactions of these complexes with biomembranes helps to elucidate some steps of DV life cycle.

P-040

The aggregation of amphotericin B in the lipid membranes induced by K^+ and Na^+ ions: Langmuir monolayers study

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The polyene antibiotic amphotericin B (AmB) is currently the drug of choice in the treatment of fungal infections despite its undesirable side effects. According to the general conviction, the biological action of the drug is based on the formation of transmembrane channels which affect physiological ion transport, especially K^+ ions. This work reports the results of Langmuir monolayers study of the effect of K^+ and Na^+ ions on the molecular organizations of AmB in the model lipid membrane. The two-component monolayers containing AmB and phospholipid (DPPC) have been investigated by recording surface pressure-area isotherms spread on aqueous buffers containing physiological concentration of K^+ and Na^+ ions. The strength of the AmB-DPPC interactions and the stability of the mixed monolayers were examined on the basis of surface pressure measurements, the compressional modulus and the excess free energy of mixing. The obtained results proved a high affinity of AmB towards lipids in the presence of K^+ than Na^+ ions. The most stable mixed monolayers were formed with the 1:1 and 2:1 stoichiometry in the presence of K^+ and Na^+ ions, respectively.

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P-041

Microcalorimetric study of antibiotic amphotericin B complexes with Na^+, K^+ and Cu^{2+} ions

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Amphotericin B (AmB) as a metabolite of Streptomyces nodosus is one of the main polyene antibiotics applied in the treatment of deep-seated mycotic infections. We presented microcalorimetric (DSC) study of molecular organization of amphotericin B in lipid membranes induced by Na^+, K^+ and Cu^{2+} ions. The analysis of DSC curves indicates the influence of $Na^+$and K^+ ions on the main phase transition of pure DPPC lipid. For the molar fractions of 3, 5, 10, 15 mol% AmB in DPPC we observed the thermal shift towards higher temperatures in respect to pure lipid, both in the presence of Na^+ and K^+ ions. This result may be connected with the changes in dynamic properties of the model membrane system. In case of AmB-Cu^{2+} complexes in aqueous solution at two pH values, 2.0 and 10.6, the DSC measurements reported endothermic heat effect. This phase transition was related to the dissociation process of AmB-Cu^{2+} complexes. The formation of AmB-Cu^{2+} complexes are accompanied by changes of the molecular organization of AmB especially disaggregation. These all observed effects might be significant from a medical point of view.

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P-042

GSH-Ferrocene conjugates as electrochemical sensors for gluthatione S-transferases

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Enzymes belonging to the glutathione S-transferase (GST) family catalyze the conjugation of glutathione (GSH) to different compounds, mainly xenobiotics, so that they can be excreted from the cell. Over-expression of GSTs was demonstrated in a number of different human cancer cells associated with the resistance to many anticancer chemotherapeutics. Thus, a quick and specific determination of GST levels would be very helpful in cancer diagnosis. Voltammetric techniques are simple, sensitive and suitable for real time monitoring of chemical and biological reactions, but cannot be used to examine the GST-GSH interactions. However, it is known that metallocenes such as ferrocene (Fc) are neutral, chemically stable and nontoxic compounds with excellent redox properties. They have found applications in different fields as biosensors. GSH-ferrocene conjugates could be of particular interest as their reversible and tunable redox properties could be applied for the development of sensors for the electrochemical detection of GST. To evaluate the potential of GSH-ferrocene conjugates as redox probes, we have synthesized two conjugates, GSFc and GSFcGS, and have performed thermodynamic studies with the dimeric GST of *Schistosoma japonicum* (SjGST) and the two mentioned ferrocenyl conjugates along with oxidized glutathione. All of them are competitive inhibitors of SjGST and the voltammetric properties of the ferrocene-SG conjugates were examined. Our results show that the GSFcGS conjugate exhibits an affinity for SjGST approximately two orders of magnitude higher than the other two, and three orders higher than GSH alone. Furthermore, it shows negative cooperativity with the affinity for the second site two orders of magnitude lower than that for the first one. We propose the reason for this negative cooperativity is steric since our docking studies have shown that, when bound, part of the first bound ligand invades the second site due to its large size. We suggest the combination of its *quasi* 1:1 stoichiometry, enhanced voltammetric signal and high affinity makes GSFcGS a good redox probe for the electrochemical detection of GST levels.

Acknowledgments

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O-43

Crowding of membrane proteins and peptides

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Membrane proteins and peptides are acting in an environment rich in other proteins or peptides. Aim of our study was to understand how such molecular crowding and resulting intermolecular interactions can influence the behavior of membrane proteins, using various antimicrobial peptides and membrane proteins as examples. In the case of antimicrobial peptides we have previously described a change in their alignment in the membrane at a characteristic threshold concentration. To understand whether this change is due to unspecific crowding or specific peptide-peptide interactions, we tested if this re-alignment depends on the presence of additional peptides. In most cases we found a similar re-orientation behavior irrespective of the added peptide type, indicating unspecific crowding. When pairing PGLa and magainin-2, however, we observed a distinctly different sequence of PGLa re-orientation in the membrane, indicating a specific interaction between these two peptides, which correlates well with their known synergistic activity.

A rather different effect of crowding was observed for the larger channel protein MscL, which was found to form clusters of functionally active proteins in the membrane. We propose that this clustering is caused by lipid-mediated protein-protein interactions.

P-044

Water, hydrophobic interaction, and protein stability

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Although there are several forces maintaining protein structure, it is well know that hydrophobic interaction is the dominant force of protein folding. Then, we can infer that any factor that alters hydrophobic interaction will affect the protein stability. We have study by computer simulation a model system consisting in solution of Lenard-Jones particles in water (SPC/E model) at different pressures and temperatures and analyzed the solubility *i.e.* the

aggregation properties, of such a system. From the obtained data we are able to build up the phase surface determining the critical point. The computing results where compared with experimental data of binary mix of non polar substance in water and of protein denaturation, finding high coincidence on the critical point. Since the behavior of our model system can only be due to hydrophobic effects, the coincidences with the denaturation of proteins allow us to conclude that the dominant factor that determine temperature and pressure denaturation of proteins is the hydrophobic interaction. The temperature and pressures at which the denaturation, as well the disaggregation of simple non-polar particles, starts agree with what we could expect based on the cross over line of the low to high density structure water transition.

P-045
Ligand induced changes of a functionally reconstituted membrane protein in a supported lipid bilayer

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The functional reconstitution of a mitochondrial membrane protein into a lipid bilayer was studied using a quartz crystal microbalance. The 6xHis-tagged protein was immobilised *via* specific binding to a Cu^{2+} terminated sensor surface, with a change in frequency indicating approximately 75% coverage of the sensor surface by the protein. A lipid bilayer was reconstituted around the protein layer, with a final change in frequency that is consistent with the remaining area being filled by lipid. Incubation with a specific ligand for the protein resulted in a significant change in frequency compared to the interaction with the surface or lipid alone. The change is greater than expected for the mass of the ligand, indicating a possible conformational change of the protein, such as the opening of a channel and increased water content of the layer. Electrical impedance measurements on the same system have provided additional evidence of protein-lipid bilayer formation, and it is intended that this system will be studied with neutron reflectometry to characterise potential ligand induced channel formation. Valuable functional and structural information about this membrane protein was obtained by using surface sensitive techniques to study the protein in a biomimetic lipid bilayer.

O-046
Visualizing and quantifying HIV-host interactions with fluorescence microscopy

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Protein-chromatin interactions are classically studied with in vitro assays that only provide a static picture of chromatin binding. Fluorescence correlation spectroscopy (FCS) is a non-invasive technique that can be used for the same purpose. Being applicable inside living cells it provides dynamic real-time information on chromatin interactions. Transcriptional co-activator LEDGF/p75 has well characterized protein and chromatin interacting regions. We studied LEDGF/p75 in vitro and inside living cells with FCS and other techniques (luminescent proximity assay, spot/half-nucleus fluorescence recovery after photobleaching, continuous photobleaching). Protein-protein interactions in living cells can be monitored with fluorescence cross-correlation spectroscopy (FCCS) using fluorescent proteins as genetic labels. Advantages over using Förster resonance energy transfer (FRET) are the independence from intermolecular distance and knowledge of absolute protein concentrations. We characterized FCCS with fluorescent proteins in vitro and then studied the intracellular complex of LEDGF/p75 and the HIV-1 integrase (IN) enzyme both with FRET and FCCS.

P-047
Do nucleolar and apoptotic proteins interact in actinomycin D-treated cells?

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Nucleus and its compartment nucleolus are a seat of enormous biosynthetic activity in human cancer cells. Nucleolar proteins, e.g. B23 or C23, play an important role in regulation of cell division and proliferation. One of the strategies how to intermit malignant cell proliferation is affecting, e.g. by drug treatment, a net of intracellular protein interactions to bring the cell on a way of apoptosis. A cytostatic agent actinomycin D initiates apoptosis in human cancer cells, as well as in normal peripheral blood lymphocytes. At the same time, translocation of B23 and C23 into nucleoplasm is observed in the treated cells. Therefore interaction between nucleolar and apoptotic proteins comes into a question. Co-immunoprecipitation, fluorescence microscopy and yeast two hybrid analysis are used to answer it. In co-immunoprecipitation experiments, tumor suppressor p53 showed up to be a promising candidate for the interaction. Fluorescence

microscopy showed that actinomycin D-stabilized p53 protein is localized mainly in nucleoplasm and therefore there is a possibility of its interaction with the relocalized nucleolar proteins. The yeast two-hybrid system is used for screening cDNA library with the baits of interest and also for further testing of possibly interacting candidates.
This work was supported by the grants IGA NS 9637-4 and IGA VZ00023736 from the MHCR

P-049

Anisotropy study of OK kidney cells in the presence of aminoglycoside gentamicin

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It is known that gentamicin causes nephrotoxicity by inhibition of protein synthesis in renal cells. This causes necrosis of proximal tubular cells, leading to acute renal failure. The aim of this study was to investigate the membrane fluidity of "*Opossum kidney*" (OK) cells treated with gentamicin at different temperatures in order to establish the capacity of gentamicin to influence the packing of membrane lipids. For determination of membrane fluidity, OK cells were incubated with 1mM gentamicin. Dependence of membrane fluidity with temperature was recorded by measuring the fluorescence depolarization of TMA-DPH incubated cells, in the temperature range between 10^0 - 40°C. The anisotropy of OK cells treated with gentamicin is higher than in control cells at temperatures above 35°C **(**t- Student test showed that the differences become significant at temperatures higher than 35°C). This may be due to loss of lipids packing above this temperature which favors a higher exposure of negative lipid polar heads to the gentamicin positive charges and a more effective electrical action of gentamicin on each negatively charged membrane constituent. In conclusion, gentamicin induces a significant increase in membrane fluidity in OK renal cells at temperatures above 35°C.

P-050

Thermodynamics: a tool for affinity optimization of drug candidates for transthyretin amyloidosis

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Among the pathological conditions associated with amyloid fibrils, familial amyloid polyneuropathies (FAP) exhibit fibril deposits mostly constituted by variants of Transthyretin (TTR), a homotetrameric plasma protein implicated in the transport of thyroxine and retinol [1].
Nowadays, the only effective therapy for TTR amyloidosis is liver transplantation. New therapeutic strategies are being developed taking advantage of our current understanding of the molecular mechanisms of amyloid formation by TTR [2]. A significant effort has been devoted to the search and rational design of compounds that might decrease TTR tetramer dissociation, for example, through ligand binding at the thyroxine binding sites of TTR [3, 4].
Here, we use isothermal titration calorimetry (ITC) to characterize the thermodynamic binding signature of potential TTR tetramer stabilizers, previously predicted by computer-assisted methods [3]. ITC allows the measurement of the magnitude of the binding affinity, but also affords the characterization of the thermodynamic binding profile of a protein-ligand interaction. High affinity/specificity TTR ligands, enthalpically and entropically optimized, may provide effective leads for the development of new and more effective drug candidates against TTR amyloidosis.

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P-051

Fluorescence lifetime as a tool to study protein-protein interactions of GroEL and PRD1 viral proteins

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We have established a set of vectors to promote easy cloning of eCFP and eYFP fusions with any protein of interest. We exploit these fluorescent fusion proteins to study protein-protein interactions by fluorescence lifetime of eCFP. The decrease of eCFP lifetime reveals FRET between eCFP and eYFP and hence the interaction between proteins in question.
GroEL-GroES chaperonin complex is required for the proper folding of *Eschericia coli* proteins. Bacteriophage T4 and its distant relative coliphage RB49 encode co-chaperon proteins (respectively gp31 and CocO) that can replace GroES in the chaperonin complex. GP31 is also required in the folding of the major capsid protein of the phage. PRD1 is a large membrane-containing bacteriophage infecting gram-negative

bacteria such as *E. coli* and *Salmonella enterica*. It has 15 kb long linear dsDNA genome and the capsid has an icosahedral symmetry. The GroEL-GroES chaperonin complex is needed in the assembly of PRD1. We have found evidence that PRD1 protein P33 can work similar way as other viral co-chaperones and substitute GroES in chaperonin complex. Fluorescence lifetime studies between proteins GroEL and P33 reveals an interaction that backs up the theory.

P-052
Structural modification of model membranes by fibrillar lysozyme as reaveled by fluorescence study
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Recent experimental findings suggest that protein aggregation, leading to the formation and depositions of amyloids play a central role in the neurodegenerative diseases, type II diabetes, systemic amyloidosis, etc. In the present study we focused our efforts on investigation of the influence of fibrillar lysozyme on the structural state of model lipid membranes composed of phosphatidylcholine and its mixtures with cardiolipin (10 mol %) and cholesterol (30 mol %). To achieve this purpose, two fluorescent probes with different bilayer location, 1,6-diphenyl-1,3,5-hexatriene (DPH) distributing in membrane hydrocarbon core and 6-lauroyl-2-dimethylaminonaphthalene (Laurdan) locating at lipid-water interface, have been employed. The changes in membrane viscosity under the influence of amyloid lysozyme were characterized by fluorescence anisotropy of DPH. This fluorescence parameter was not markedly affected by fibrillar protein in all types of model membranes. The changes in emission spectra of Laurdan were analysed by the generalized polarization value (GP). It was found that adding of amyloid lysozyme resulted in the increment of GP value. Our data suggest that lysozyme fibrils cause reduction of bilayer polarity and increase of lipid packing density.

P-053
A new ITC assay for measuring ultratight and low-affinity protein–ligand interactions
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Isothermal titration calorimetry (ITC) is the gold standard for the quantitative characterisation of protein–ligand and protein–protein interactions. However, reliable determination of the dissociation constant (K_D) is typically limited to the range 100 $\mu M > K_D > 1$ nm. Nevertheless, interactions characterised by a higher or lower K_D can be assessed indirectly, provided that a suitable competitive ligand is available whose K_D falls within the directly accessible window. Unfortunately, the established competitive ITC assay requires that the high-affinity ligand be soluble at high concentrations in aqueous buffer containing only minimal amounts of organic solvent. This poses serious problems when studying protein binding of small-molecule ligands taken from compound libraries dissolved in organic solvents, as is usually the case during screening or drug development. Here we introduce a new ITC competition assay that overcomes this limitation, thus allowing for a precise thermodynamic description of high- and low-affinity protein–ligand interactions involving poorly water-soluble compounds. We discuss the theoretical background of the approach and demonstrate some practical applications using examples of both high- and low-affinity protein–ligand interactions.

P-054
Interaction of myoglobin with oxidized polystyrene surfaces studied using rotating particles probe
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The interaction of proteins with polymer surfaces is of profound importance for the sensitivity of biosensors. Polymer surfaces are often treated in order to tune their chemical and physical properties, for example by oxidation processes. To get a better understanding of the association of proteins to treated polymer surfaces, we use the rotating particles probe (X.J.A. Janssen et al., Colloids and Surfaces A, vol. 373, p. 88, 2011). In this novel technique, protein coated magnetic particles are in contact with a substrate and the binding is recorded for all individual particles using a rotating magnetic field. We investigate the interaction of myoglobin coated magnetic particles to spincoated polystyrene surfaces that have been oxidized with a UV/ozone treatment. The surfaces have been characterized by XPS, AFM and water contact angle measurements. We will demonstrate a clear influence of polystyrene oxidation on the binding fractions of the myoglobin coated particles. We interpret the results in terms of DLVO-theory: electrostatic as well as electrodynamic properties of the surfaces will be influenced by the oxidation.

P-055
The effects of mouse twinfilin-1 on the structure and dynamics properties of actin
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The actin cytoskeleton of eukaryotic cells plays a key role in many processes. The structure and dynamics of the cytoskeleton are regulated by a large number of proteins that

interact with monomeric and/or filamentous actins. Twinfilin is a 37-40 kDa protein composed of two ADF-homologue domains connected by a short linker.
In our work we studied the effects of the mouse twinfilin-1 (TWF1) on the monomeric actin. We determined the affinity of TWF1 to the ATP-actin monomer with fluorescence anisotropy measurement (K_D = 0.015 μM). The fluorescence of the actin bound ε-ATP was quenched with acrylamide in the presence or absence of TWF1. In the presence of twinfilin the accessibility of the bound ε-ATP decreased, which indicates that the nucleotide binding cleft is shifted to a more closed conformational state. It was confirmed with stopped-flow experiments that the kinetics of nucleotide-exchange of actin decreased in the presence of TWF1. We determined the thermodynamic properties of TWF1 and investigated the effect of twinfilin on the stability of actin monomer with differential scanning calorimetry. The TWF1 stabilized the stucture of the G-actin. Our results can help us to understand the regulation of actin cytoskeleton in more details.

P-056
Nephrotoxicity of i.v. administered oleic acid coated Fe_3O_4 nanoparticles (NP) in rats
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Magnetic NP have attracted attention due to their potential of contrast enhancement of magnetic resonance imaging and targeted drug delivery, e.g. tumor magnetic hyperthermia therapy. Potential nephrotoxicity of single i.v. administration of Fe_3O_4 NP was studied in female Wistar rats i.v. administered either placebo (10% v/v rat serum in 0.9% NaCl), suspension of TiO_2 NP (positive control, bimodal 84/213 nm distribution), or Fe_3O_4 NP (bimodal 31/122 nm distribution) in doses of 0.1, 1.0 or 10.0 mg/kg. Rats were sacrificed 24h, 7-, 14- and 28-days after NP injection (n=9-10/each group). Administration of NP did not alter kidney size significantly; renal function of NP administered rats as monitored by plasma creatinine and urea concentrations, creatinine clearance and protein excretion rate did not differ significantly in either interval from rats administered placebo. One week after administration significant rise in plasma Ca, its urinary and fractional excretion was observed in rats administered 10 mg Fe_3O_4/kg. Plasma Mg levels rose in this group 1 and 2 weeks after administration. No significant changes in the expression of TNF-α, TGF-β, and collagen IV genes in renal cortex were revealed. No obvious nephrotoxic effects were observed in rats after a single i.v. dose of Fe_3O_4 NP.
Study was supported by 7FP EC EU: NanoTEST (Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics.), Grant No.: 201335.

P-057
Biomimetic supramolecular assemblies for studying membrane interactions in vitro and in vivo
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We designed a novel biomimetic sensor, composed of conjugated polydiacetylene (PDA) matrix embedded within lipid vesicles. The system is capable of detecting various compounds occurring within lipid membranes through rapid colorimetric as well as fluorescent transitions. The colorimetric response of the sensor is correlated to the extent of compound-membrane binding and permeation and quantified binding sensitivity to lipid composition. We describe a new disease diagnostic approach, denoted *"reactomics"*, based upon reactions between blood sera and an array of vesicles comprising different lipids and polydiacetylene (PDA), a chromatic polymer. We show that reactions between sera and such a lipid/PDA vesicle array produce chromatic patterns which depend both upon the sera composition as well as the specific lipid constituents within the vesicles. Through attachment of chromatic polydiacetylene (PDA) nanopatches onto the plasma membrane, real-time visualization of surface processes in living cells is possible.

P-058
Mechanistic studies of the Ras-superfamily by time-resolved FTIR spectroscopy
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The Ras protein is mutated in 30% of human tumors. Ras acts as a switch, transmitting a growth signal in an active GTP-bound form and turning the signal off in an inactive GDP-bound form. The switch off is accomplished by GTP hydrolysis, which is catalyzed by Ras and can be further accelerated by GTPase activating proteins (GAPs). Mutations which prevent hydrolysis cause severe diseases including cancer.
We investigate the reaction of the Ras GAP protein-protein complex by time-resolved FTIR spectroscopy.[1] Detailed information on the mechanism and the thermodynamics of the reaction was revealed:[2] First, the catalytic arginine-finger of GAP has to move into the GTP binding pocket, then cleavage of GTP is fast and H_2PO_4 hydrogen-bonded in an eclipsed conformation to the β-phosphate of GDP is formed.
Further, we performed for the first time ATR-FTIR spectroscopy of Ras in its native environment, a lipid membrane.[3] In this setup we are able to do difference spectroscopy of the immobilized protein. Interactions with other proteins can be determined in a similar way as in SPR experiments but with the additional information from the infrared spectra.

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2. Kötting et al. *PNAS* **105**, 6260 (2008).
3. Güldenhaupt et al., *FEBS J.* **275** 5910 (2008).

P-059

ATR-FTIR Spectroscopy of immobilized proteins

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We report on trFTIR[1] investigations on the Ras protein, which is mutated in 30% of the human tumors. Ras proteins undergo distinct post-translational lipid modifications. These are required for appropriate membrane targeting. While biological function of Ras *in vivo* is always located at a membrane, most *in vitro* studies have been conducted without the lipid anchor in solution.
We performed for the first time ATR-FTIR spectroscopy of membrane anchored Ras:[2] A monolayer of a model membrane was tethered to a germanium ATR-crystal. Subsequently Ras is immobilized at the membrane. This can be done with bioorganically synthesized lipidated Ras (H. Waldmann)[5] or by protein tags. We compare the differently membrane bound Ras proteins among themselves and with Ras is solution.
Since our setup includes a flow-through cell, we are able to do difference spectroscopy of the immobilized protein, e.g. by exchanging the nucleotide. By this method we can observe the absorption of individual functional groups. Further, the interactions with other proteins can be determined in a similar way as in SPR experiments but with the additional information from the infrared spectra. Since immobilization is possible e.g. with a His-tag our system can be used universal.

1. Kötting, Gerwert, *ChemPhysChem* **6**, 881 (2005).
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P-060

Carbohydrate recognition domains play different roles in galectin function

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Galectins are a family of animal lectins that specifically bind β-galactosides and have gained much attention due to their involvement in several biologic processes such as inflammation, cell adhesion and metastasis. In such processes, several issues are still not clear including the mechanisms of interaction with different carbohydrates. Galectin-4 (Gal-4) is a tandem-repeat type galectin that contains two carbohydrate recognition domains (CRD-I and CRD-II) connected by a linking peptide. In this study, we performed spectroscopic studies of the carbohydrate-recognition domains from human Gal-4. Our goals are two-fold: (1) to monitor conformational changes in each domain upon its binding to specific ligands and then to correlate the observed changes with structural differences between the CRDs and (2) to investigate the interaction between the CRDs and lipid model membranes. To achieve such objectives we used a combined approach of spectroscopic techniques involving Circular Dichroism and Electron Spin Resonance. Overall the results obtained so far show that CRD-I and CRD-II have distinct behaviors in terms of carbohydrate recognition and membrane binding. This may be due to specific differences in their structures and certainly suggests a non-equivalent role in protein function.

P-061

Hemoglobin influence on lipid bilayer structure as revealed by fluorescence probe study

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Hemoglobin (Hb) is a red blood cell protein responsible for the oxygen transport. Its affinity for lipid bilayers represents interest for gaining insight into protein biological function as well as for some applied aspects such as development of blood substitutes or biosensors. Hb influence on lipid bilayer structure was investigated using fluorescent probes pyrene and Prodan. Model membranes were prepared of phosphatidylcholine (PC) and its mixtures with phosphatidylglycerol (PG) and cholesterol (Chol). Hb penetration into membrane interior is followed by the increase of relative intensity of pyrene vibronic bands and decrease of Prodan general polarization value suggesting an enhancement of bilayer polarity. This implies that Hb incorporation into membrane interior decreases packing density of lipid molecules, promoting water penetration into membrane core. Chol condensing effect on lipid bilayer prevents protein embedment into bilayer, thus decreasing membrane hydration changes as compared to PC bilayers. In the presence of anionic lipid PG Hb-induced increase of bilayer polarity was found to be most pronounced, pointing to the modulatory role of membrane composition in Hb bilayer-modifying propensity.

P-063

Thermodynamic evaluation of high affinity CD22 antagonists

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CD22, expressed on the surface of B cells, is a member of the sialic-acid binding immunoglobulin-like lectin (Siglec) family. It is a negative regulator of B cell signaling and plays a crucial role in the regulation of activity, homeostasis and survival of B cells [1]. Furthermore, the presence of cytoplasmic CD22 was proven to be a marker in B cell malignancies [2]. The physiological ligands of CD22 are α2,6-linked sialic acids on glycoproteins and gangliosides. The minimal binding motif is Siaα2,6-Gal (or Gal*N*Ac, Glc*N*Ac) [3].

We present optimized sialic acid-based mimics binding in the low nanomolar range. Molecular interactions were determined with surface plasmon resonance (SPR), characterizing the affinity and the kinetics of binding. Furthermore, isothermal titration calorimetry (ITC) was applied to dissect the standard free energy of binding (ΔG°) into the standard enthalpy of binding (ΔH°) and the standard entropy of binding (ΔS°).

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P-064

Importance of the low enantioselectivity of PGK for the phosphorylation of antiviral L-nucleosides

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D- and L-nucleoside analogues are compounds of clinical interest in antiviral therapies. In order to pass the cell membranes, most of these medicines has to be administrated to patients as nucleoside pro-drugs and not directly as triphosphorylated forms. Because of the poor phosphorylation of the nucleoside analogues used in therapy, it is important to understand and to optimize their metabolism. Our aim is to understand how compounds of chirality L turn away 3-phosphoglycerate kinase (PGK) from its normal glycolytic function to be converted into the triphosphate forms. In order to elucidate PGK mechanism and substrate specificity, we have measured the kinetics of the different steps of the enzymatic pathways by rapid mixing techniques and studied the influence of the nature of the nucleotide substrate thereon. We first performed an extensive study with D- and L-ADP (see poster by P. Lallemand). We are now extending the studies to other nucleotide diphosphates (some of them used in therapies). Changes in the nature of the nucleobase or deletion of hydroxyl group of the sugar affect the efficiency of phosphorylation by PGK, either by decreasing dramatically their affinity or by altering the phospho-transfer step itself. Structural explanations are given based on docking data.

P-065

New insights into the translocation route of enrofloxacin and its metalloantibiotics

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Probing drug/lipid interactions at the molecular level represents an important challenge in pharmaceutical research, drug discovery and membrane biophysics. Previous studies showed that enrofloxacin metalloantibiotic has potential as an antimicrobial agent candidate, since it exhibits antimicrobial effect comparable to that of free enrofloxacin but a different translocation route. These differences in uptake mechanism can be paramount in counteracting bacterial resistance. In view of lipids role in bacterial drug uptake, the interaction of these compounds with different E. coli model membranes were studied by fluorescence spectroscopy. Partition coefficients determined showed that lipid/antibiotic interactions were sensitive to liposomes composition and that the metalloantibiotic had a higher partition than free enrofloxacin. These results corroborate the different mechanism of entry proposed and can be rationalized on the basis that an electrostatic interaction between the metalloantibiotic positively charged species, present at physiological pH, and the lipids negatively charged head groups clearly promotes the lipid/antimicrobial association.

P-066

Oligomerization and fibril assembly of amyloid β peptide (1-40) in the presence of amphiphiles

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Amyloid β peptide (Aβ) forms a large amount of extracellular deposits in the brain of Alzheimer's disease (AD) patients and it is believed that this peptide is related to the pathogenesis of that disease. The most abundant monomeric form of physiological Aβ ($\sim$90%) is constituted by 40 amino acids and is benign, but by an unknown mechanism this endogenous material becomes aggregated and neurotoxic. Increasing evidence suggests that membrane interaction plays an important role in Aβ neurotoxicity.
In this work it will be studied the interactions of Aβ(1-40) with CTAC (cationic), SDS (anionic), PFOA (anionic with fluorine atoms) and OG (nonionic) amphiphiles in monomeric and micellar forms.
The results demonstrated that Aβ(1-40) forms fibrils with different morphologies in the presence of micelles. In addition, the presence of micelles accelerates the formation of fibrils and decreases the lifetime of oligomers.

P-067

On chip multiplex label free bio-affinity analysis and MALDI-MS characterisation of bound analyte

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We present here the exploitation of the powerful approach of Surface Plasmon Resonance imaging and Mass Spectrometry coupling for protein fishing in biological fluids such as human plasma at the same sensitivity. On one hand, multiplex format SPRi analysis allows direct visualization and thermodynamic analysis of molecular avidity, and is advantageously used for ligand-fishing of captured bio-molecules on multiple immobilized receptors on a SPRi-Biochip surface. On the other hand, MALDI mass spectrometry is a powerful tool for identification and characterization of molecules captured on specific surface. Therefore, the combination of SPRi and MS into one concerted procedure, using a unique dedicated surface, is of a great interest for functional and structural analysis at low femtomole level of bound molecules.
To reach these goals, particular surface engineering has been engaged to maintain a high level of antibody grafting and reduce non-specific adsorption. Thus, various chemistries have been tested and validated towards biological fluids such plasma, keeping in mind the capacity of the in situ investigation by MS.
Finally, signal to noise ratio was magnified leading to the characterization of protein LAG3, a potential marker of breast cancer, in human plasma.

P-069

Atenolol incorporation into PNIPA nanoparticles investigated by Isothermal Titration Calorimetry

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Poly(N-isopropylacrylamide) (PNIPA) is a thermo-sensitive hydrogel undergoing a volume phase transition at about of 32°C close to the body temperature. This volume phase transition is envisaged as a key property for drug binding and release.
The purpose of our research is the thermodynamic characterization of the binding of atenolol by PNIPA polymeric nanoparticles. The thermodynamic parameters which characterize the binding process are obtained using the Isothermal Titration Calorimetry (ITC) as the main investigation technique.
When polymeric nanoparticles bind drug molecules, heat is either generated or absorbed depending on the amount of bond molecules and also on the exothermic or endothermic character of the binding process. The heat measurement allows the determination of binding constants, reaction stoichiometry and the thermodynamic profile of the interaction. ITC technique has been used to investigate the binding properties of nanoparticles which shrink from the swollen to the collapsed state. The capacity of such nanogels to bind atenolol molecules is directly related to relevant differences between the binding properties in the swollen and in the collapsed state respectively.

P-070

Aggregation study of ω-(alkyldimethylamonium)-alkylaldonamide bromides

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Sugar-based surfactants are of considerable research interest because they have improved surface and performance properties, reduced environmental impact, and have potential pharmaceutical and biomedical applications. ω-(alkyldimethylamonium)alkylaldonamide bromides (C_nGAB) with different chain lenghs ($n = 10$, 12, 14, 16) belonging to cationic sugar-based surfactants were newly synthesised. The aggregation processes of C_nGABs were studied by means of isothermal titration calorimetry (ITC), electric conductance method and molecular modelling methods. The critical micelle concentrations (cmc), the degree of micelle ionization (β), the enthalpies (ΔH_m) and the entropies (ΔS_m) of micellization as well as the contributions of the headgroups to the Gibbs free energies (ΔG^0_m(hy)) were calculated. The obtained values were compared with those for dodecyldimethylethylammonium bromide and literature data for analogical glucocationic surfactants. The latest compounds differ from C_nGAB surfactants by substitution of sugar chain by gluco ring. Molecular modelling methods were used to relate the molecular properties of the compounds with their experimentally studied properties in solution. This work was supported by grant N N305 361739.

P-071

Characterization of membrane active regions of dengue virus (DENV) proteins C and E

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Every year over 50 million people are infected with dengue virus (DENV), transmitted by a mosquito (*Aedes aegypti*). This enveloped virus, member of the *Flaviviridae* family, has four distinct serotypes. It has a single stranded positive RNA molecule with a single open reading frame that encodes a single poliprotein, which, after appropriate processing by viral and host proteases, gives rise to three structural proteins (C, prM and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [1]. The surface of the immature virion is composed of E and prM heterodimers that are arranged as trimers protruding from the membrane [2]. The virus is thought to enter the host cell via a receptor-

mediated endocytosis, although, if any, the specific dengue receptors have not been described. Once inside the cell, the acidified environment inside the endocytic vesicle triggers an irreversible trimerization of the envelope (E) protein, inducing the release of the nucleocapsid (composed of viral RNA and multiple copies of C protein) to the cytoplasm, thus starting the infection process, where the poliprotein is translated and processed, originating all viral proteins. Considering the structural proteins C and E, these are essential for the viral infection, specifically, protein C is thought to be involved in the viral assembly and specific encapsidation of the genome and protein E (a class II fusion protein) plays a major role in the fusion process. As recently described by some studies [3], protein C is composed of four α helices connected by four short loops and has a highly hydrophobic region forming a concave groove that could interact with lipid membranes and a region with an increased concentration of positive charges, possibly interacting with the viral RNA. As for protein E, it is composed of three β stranded domains. It is proposed that the fusion loop is located in domain II of this protein and the putative receptor binding sites, considered essential for the viral entry, are supposedly located in domain III. In this work, we describe the identification of the membrane active regions of both these proteins, considering both theoretical studies, hydrophobic moments, hydrophobicity and interfaciality values as well as experimental ones, namely fluorescence spectroscopy, where a fluorescent probe is encapsulated in model membrane systems, and differential scanning calorimetry [4]. We have found one region in protein C and four regions in protein E with membranotropic activity. This is the first work describing experimentally the putative membrane interacting zones of both these proteins.

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P-072
Investigation of membrane-membrane interaction mediated by coiled coil lipopeptides
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Specific cellular membrane interaction and fusion are crucial points *in vivo* which are in eukaryotic cells mediated by SNARE proteins. The definite mechanism behind these processes is still poorly understood, but the coiled coil formation of a SNARE core complex consisting of four α-helices seems to generate a fusogenic driving force. This offers the possibility to design a straightforward experimental setup to mimic the complex protein-mediated membrane-membrane interaction by using mere protein fragments or peptides attached to artificial lipid bilayers which self-assemble to a coiled coil structure.
In our approach, two artificial three heptad repeat coiled coil forming peptides were synthesized and attached to maleimide functionalized membranes via an *in situ*-coupling reaction. Thus, secondary structure changes, kinetic characteristics and binding energetics were monitored during coiled coil formation with real time ellipsometry, IR and CD spectroscopy. The lipopeptide mediated membrane-membrane interaction itself is investigated by colloidal probe spectroscopy and TIRFM.
These techniques and the setup of our model system allow screening the energetic and structural properties of variable coiled coil forming peptides, i.e. linker-modified or biologically inspired sequences.

O-073
Enzymatic reactions in nanostructured surfaces: unzipping and cutting the double helix
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Protein-DNA interactions are vital for living organisms. From viruses to plants and humans, the interactions of these two different classes of biopolymers control processes as important and diverse as the expression of genes and the replication, rearrangement, and repair of DNA itself.
To understand these processes at the molecular level, and to follow changes in cellular pathways due to different kinds of perturbations and/or diseases it is necessary the identification and quantification of proteins and their complex network of interactions.
We have exploited the high spatial resolution given by Atomic Force Microscopy to generate DNA arrays of variable density by means of Nanografting. On such nanostructures, we investigate the mechanism of different enzymatic reactions (from restriction enzymes to helicases).
Registering with high precision the height variation due to the action of the enzyme onto the engineered DNA sequences (in the case of restriction enzymes) or taking advantage of the different mechanical properties of single and double stranded DNA (in the case of helicases, where for the first time kinetic data were obtained on RecQ1 human helicase), we were able to monitor either the activity and/or the action mechanisms of these two important classes of enzymes.

P-074
Electric hyperfine interactions ab initio study in biological systems: Cd in DNA bases
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Electric hyperfine interactions can be a powerful tool to identify local environments in many different systems.

Recently it has been drawn attention to the possible application of the Time Differential Perturbed Angular Correlation (TDPAC) technique to study differences in the Nuclear Quadrupole Coupling constants (υQ) at Cd probes in mouse DNA infected with the *Trypanosoma Cruzi* (Chagas disease vector). The electric contribution to the hyperfine interaction is usually expressed as the nuclear quadrupole coupling frequency υQ which is given by the product of the nuclear quadrupole moment Q and the Electric Field Gradient (EFG) at the nucleus. The EFG can be theoretically obtained from an *ab initio* electronic structure calculation. The 111In → 111Cd γ-γ decay can be used in a TDPAC measurement to investigate the Cd metal binding to DNA. The interaction of the metal with the DNA bases can change many aspects of the base pairing [1]. Here we study electric hyperfine properties of Cd bound to some DNA bases. The methodology used for the electronic structure calculations is based on the Kohn Sham [2] scheme of the Density Functional Theory (DFT) and the Car-Parrinello [3] method. We use the Projector Augmented Wave [4] method as embodied in the CP-PAW computational code. The results of EFG and energies are discussed as function of water molecules present in the Cd^2+ environment and compared with TDPAC measurements at Cd probes in mouse DNA infected with the *Trypanosoma Cruzi.* The study of Cd2+ interactions with DNA has also a very broad interest due to Cadmium-induced carcinogenesis.

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P-075

Structure and stability of cyclic peptide based nanotubes: a computational chemistry investigation

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In this study an attempt has been made to investigate the structure, dynamics and stability of cyclic peptide nanotubes (CPNTs) formed by the self-assembly of cyclic peptides (CPs) using classical molecular dynamics (MD) simulation and semiempirical quantum chemistry calculation employing PM6. The structure and energetics of monomer and various oligomeric CPNTs have been investigated by considering the (*cyclo*-[(D-Ala-L-Ala)$_4$]) peptide as the model for CP. Various geometrical parameters extracted from the MD simulation reveal that the terminal residues are loosely hydrogen bonded to the inner subunits regardless of degree of oligomerization. The hydrogen bonds present in the inner core regions are stronger than the terminal residues. As the degree of oligomerization increases, the stability of the tube increases due to the hydrogen bonding and stacking interactions between the subunits. The results show that the binding free energy increases with the extent of oligomerization and reaches saturation beyond CPNT5. In addition, hydrophobic and electrostatic interactions play crucial roles in the formation of CPNTs. Analysis of both structural and energetics of formation of CPNTs unveils that the self-assembly of dimer, trimer and tetramer CPNTs are the essential steps in the growth of CNPTs.

Reference

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P-076

Studies at the liquid-air interface: a powerful toolbox to study interactions between biomolecules

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Monolayers on a Langmuir trough constitute a great biomimetic model to characterize protein-protein or protein-lipid interaction, where the physical state of the interfacial layer is completely controlled. We present here three studies performed on monolayers, with a wide panel of experimental (optical, spectroscopical, rheological) techniques.
i) Surface properties and conformation of *Nephila clavipes* Spider recombinant silk proteins (MaSpI and MaSp2) was studied at the air-water interface: we show that the mechanism of assembly of both proteins is different, although both proteins share the same sequence pattern and a close hydrophobicity. They both exhibit a certain propensity to form β-sheets that may be important for the efficiency of the natural spinning process.
ii) The dystrophin molecular organization and its anchoring in a lipidic environment depend on the rod fragment used and on the lipid nature. Moreover the interaction is guided by the lateral surface pressure. This lipid packing variation is essential to understand the role of the dystrophin during compression-extension cycle of the muscle membrane.
iii) We evidence that non additive behavior of mixtures of food globular proteins leads to enhanced foaming properties or to self assembled objects.

P-078

Membrane interactions and translocation assays for nucleolar-targeting peptides

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Nucleolar-targeting peptides (NrTPs) were designed by structural dissection of crotamine, a toxin from the venom of a South-American rattlesnake. At μM concentration, NrTPs penetrate different cell types and exhibit exquisite nucleolar localization. The aim of this work was to pursue with the study of NrTPs molecular mechanism for translocation, as well as to determine the ability of NrTP to delivery large molecules into cells. For the translocation experiments, rhodamine B-labeled NrTPs were used and tested with giant multilamellar vesicles. Confocal microscopy results show that there is an efficient translocation across model membranes. High levels of intracellular peptide were also seen in different cell lines and PBMC, soon after incubation with NrTP. Furthermore, a conjugate of NrTP (NrTP6C) bound to β-galactosidase was prepared by chemical synthesis and tested in HeLa cells. This conjugate maintains enzymatic activity and is stable at 4°C for several days. The work done so far with this new family of cell-penetrating peptides revealed strong interaction and translocation with lipid model systems. Moreover, successfully cellular delivery of β-galactosidase was observed and quantified.

P-079

Interaction of zinc phthalocyanine with ionic and non ionic surfactants: UV-vis absorption and fluorescence spectroscopy for application in Photodynamic Therapy

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Among the second-generation photosensitizer (PS) developed for the treatment of neoplastic diseases by photodynamic therapy (PDT), metallo-phthalocyanines (MPc) have been proposed as an alternative to the currently used PS in clinical application. Unsubstituted MPc are not soluble in physiological solvents and their in vivo administration relies upon their incorporation into carriers or their chemical conversion into water-soluble dyes by the attachment of selected substituents. In this work, uv-vis absorption and fluorescence spectroscopy were used to study the ability of different micelles for dispersing zinc phthalocyanine (ZnPc). The following surfactants were tested: SDS, CTAB, HPS, Tween 80, Tween 20, and Pluronic F127. ZnPc has low solubility in virtually all solvents, but DMF and DMSO are observed to dissolve ZnPc in concentrations of the order of 0.9 and 0.2 mmol/L, respectively. Stock solutions of ZnPc in DMF and DMSO were prepared.

Micelles of the different surfactants containing ZnPc were prepared by dissolving in aqueous medium (milli-Q water or phosphate buffer pH 7.4) small amounts of the stock solutions previously mixed with each surfactant. The amounts of each surfactant were calculated to give an average ratio of one ZnPc molecule per micelle in the final solution. The absorption and fluorescence spectra of ZnPc in the micellar systems were obtained, and were observed to change in time. Immediately after dissolution the spectra are characteristic of monomeric ZnPc, suggesting formation ZnPc-containing nanoemulsions with the mixture of ZnPc-organic solvent in the hydrophobic region of the micelle. Since DMSO and DMF are miscible with water, the solvent diffuses out of the micelle and ZnPc stays inside the micelle in a monomeric or aggregated form. The different surfactants lead to different time evolution of ZnPc aggregation. Aggregation lifetimes vary from one hour, in the case of Pluronic F127, to more than twelve hours, in the case of CTAB and HPS. It was observed that the ionic surfactants were more efficient than non ionic ones for monomeric delivery of ZnPc .

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P-080

Nucleobase-containing peptides: a spectroscopic study of their properties and nucleic acids recognition ability

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Nucleobase-containing peptides are an important class of molecules comprising both artificial (synthetic nucleopeptides) and natural (peptidyl nucleosides and willardiine-containing peptides) compounds characterized in many cases by interesting biological properties.[1,2] In this work, we report a spectroscopic study on the properties of a chiral nucleobase-bearing peptide obtained by chemical synthesis starting from commercial sources. The findings of this research strongly encourage further efforts in the field of the use of nucleopeptides as supramolecular assembling systems and open the way to novel drug delivery approaches based on nucleobase recognition.

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P-081

Controlled immobilization and nanoscale supramolecular assembly of intrinsically disordered proteins

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Intrinsically Disordered Proteins are characterized by the lack of a well-defined 3-D structure and show high

conformational plasticity. Their structure depends tremendously on their local environment and confinment, and may accommodate several unrelated conformations, that are a strong challenge for the traditional characterizations of structure, supramolecular assembly and biorecognition phenomena. Atomic Force Microscopy (AFM) has been successfully exploited for both highly controllable nanolithography of biomolecules and for biorecognition studies, such as oriented prion protein - antibody interaction (Sanavio et al., *ACS Nano* (2010) 4(11): 6607, Bano et al. *Nano Lett* (2009) 9(7): 2614-8). Here, we report different strategies for selective, oriented confinement of alpha-synuclein at the nanoscale for sensitive and accurate direct detection, *via* precise topographic measurements on ultra-flat surfaces, of biomolecular interactions in confined assemblies.

P-082

Screening and characterization of membrane-peptide interactions in new crotamine-derived CPPs

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A new class of cell penetrating peptides (CPPs) was generated by splicing the (1-9) and (38-42) segments of crotamine, a toxin from *Crotalus durissus terrificus* venom [1]. As they localize preferably on the nucleolus, these novel CPPs were named nucleolar-targeting peptides (NrTPs). The extent of NrTP partition to zwitterionic (POPC; POPC:cholesterol 67:33) and anionic (POPG; POPC:POPG 70:30) lipid vesicles was studied following the intrinsic Tyr or Trp fluorescence of the peptides. The partition curves into POPC zwitterionic vesicles were characterized by downward slopes and higher partition coefficients ($K_p \sim 10^4$-10^5). For pure POPG, an upward curve and smaller partition coefficient point out for a different type of membrane-peptide interaction. POPC:POPG membranes present characteristics of both types of interaction. From red edge excitation shift and quenching experiments similar conclusions were attained. Leakage assays ruled out lipid vesicle disruption by crotamine or NrTPs. Further studies on NrTP cellular translocation mechanism and large molecule delivery are currently in progress.

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P-083

Modulation of the interaction and organization of Human Dystrophin repeats 11-15 subdomain with Membrane

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Dystrophin is essential to skeletal muscle function and confers resistance to the sarcolemma by interacting with cytoskeleton and membrane.
We characterized the behaviour of DYS R11-15, a five spectrin-like repeats from the central domain of human dystrophin, in the presence of liposomes and monolayers as membrane models. Interaction of DYS R11-15 depends on the lipid nature, anionic or zwitterionic, with SUVs, and on the lipid packing when comparing LUVs to SUVs. Lateral pressure of lipid monolayers modifies the protein organization and leads DYS R11-15 to form a regular network as revealed by AFM. Trypsin proteolysis assays show that the protein conformation is modified following its binding to monolayer and SUVs. Label free quantification by Nano-LC/MS-MS allowed identifying the helical amino acid sequences in repeats 12 and 13 that are involved in the interaction with anionic SUVs. Results indicate that DYS R11-15 constitutes a part of dystrophin that interacts with anionic as well as zwitterionic lipids and adapts its interaction and organization depending on lipid-packing and lipid nature.
We provide here strong experimental evidence for a physiological role of the central domain of dystrophin on sarcolemma scaffolding through modulation of lipid-protein interactions.

P-084

Structural and functional investigation of astaxanthin binding to the catalytic domain of matrix metalloprotease MMP-13

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Matrix metalloproteases (MMPs) are zinc-containing proteases involved in the remodeling and breakdown of

extracellular matrix proteins. Overexpression of the MMPs has been associated with a variety of diseases ranging from periodontal disease and arthritis to tumor invasion and metastasis. The majority of the more powerful synthetic inhibitors of MMPs incorporate a hydroxamate group, but exhibit low selectivity and are toxic. In a recent modeling study, Astaxanthin (AST), a carotenoid with potent antioxidant property, has been shown to be a potential inhibitor of MMP-13 function by occupying a binding site near the active center of the enzyme (*Bikaʹdi et al. 2006*). In our ongoing project, we investigate the binding of AST to the catalytic domain of MMPs using biochemical and ultimately crystallization to validate the proposed action of AST. Along these lines, the catalytic domain of MMP-13 (cdMMP-13) was expressed in E.coli *BL21(DE3) CodonPlus* and refolded using a novel effective refolding method. Our results reveal that AST has a potent inhibitory effect on cdMMP-13 activity, however, determination of $IC_{50\%}$ or K_i is difficult due to fast oxidation and structural instability of AST. Ongoing work aims at optimizing the inhibition conditions and improving the refolding yield to allow analyzing structure and function of the AST-bound MMP-13 in more detail.

Bikádi et al. (2006) *Bioorg Med Chem* **14**:5451-8.

P-085

Macromolecular transport across hyaluronic acid barriers

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Hyaluronic acid (Hyaluronan, HA) is a linear polysaccharide with a molar mass in the range of 10^5 to 10^7 Da and is built from alternating units of glucuronic acid and N-acetylglucosamine. Synthesized in the cellular plasma membrane, it is a network-forming and space-filling component in the extracellular matrix of animal tissues.

Here, we create hyaluronic acid films atop a porous alumina substrate, where they act as a barrier for macromolecular transport depending on their length and geometry. The geometry of the hyaluronic acid switches between a fully stretched and a mushroomlike state and is dependent on the concentration of hyaluronic acid.

To bind hyaluronic acid selectively atop the nanoporous anodic aluminum oxide (AAO), the AAO is orthogonally functionalized by silane chemistry. By means of time resolved optical waveguide spectroscopy (OWS) the transport of macromolecules, e.g. avidin, across the hyaluronic acid barriers can be recorded as a function of the pore diameter and hyaluronic acid concentration in a time resolved and label free manner. Confocal laser scanning microscopy (CLSM) provides an alternative method to investigate the orthogonal functionalization of the pores and to elucidate whether a molecule can cross the barrier at the pore entrance.

P-086

Immobilization of ABCC3 with Defined Orientation in an Environment Suitable for Transport Studies

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We functionalized gold surfaces with a hydroxy-terminated self-assembled thiol monolayer exposing an adjustable fraction of biotin moieties.[1] By *in-situ* acetylation or fluorination, surface properties could be fine-tuned to different protein immobilization scenarios. Using streptavidin as a linker protein, immobilization of human ABCC3[2] in liposomal and planar bilayer systems was possible.

ABCC3-containing proteoliposomes doped with a biotinylated anchor lipid were successfully tethered to our streptavidin-coated surfaces.

Biotinylation of the extracellular glycosylation of ABCC3 allowed direct immobilization with inside-up orientation and subsequent assembly of a lipid bilayer. Outside-up orientation was achieved by exploiting the C-terminal histidin tag of recombinant ABCC3 for immobilization via Ni^{2+} and biotin-nitrilotriacetate.

All systems were thoroughly characterized by quartz crystal microbalance, atomic force microscopy and surface plasmon resonance techniques with respect to monitoring ABCC3-mediated substrate transport in real time.

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P-089

Controversial sixth heme-ligand in non native human cytochrome c conformations

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Because of its role in the apoptotic pathway, conformational transitions of cytochrome c (cyt c) have gain on interest.

In native cyt c, Met 80 and His 18 residues serve as heme axial ligands. Cyt c interaction with the membrane causes the disruption of the iron–Met80 bond. This allows the binding of others endogenous ligands forming alternative low spin species[1,2] or induces peroxidase activity through the formation of a five coordinated high spin iron specie. Acquisition of this peroxydase activity by cyt c has been shown to be a key stage before its release from the mitochondria[3].

In order to study these non native low spin species by checking the possible amino acids able to bind the human cyt c heme, different mutants have been designed and produced: H26Q, H33N, and the double one H26Q/H33N. SDS

micelles and pH titration permit us to characterize all the possible ligands of the cyt c in the alternative low spin form. An original model of the conformational changes of micellar cyt c will be proposed.

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O-090

Unfolding of the cod parvalbumin Gad m 1 allergen by high pressure

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In countries where seafood is an integrate part of the diet, fish are among the most common food allergen sources. The major fish allergen parvalbumins are abundant in the white muscle of many fish species. Parvalbumin belongs to the family of EF-hand proteins and has a globular shape containing six helical parts.

High pressure is known to unfold proteins. We performed high pressure FTIR experiments, to explore the p-T phase diagram of cod parvalbumin, Gad m 1, to test the possibility of its inactivation by high pressure treatment.

The infrared spectrum of parvalbumin is characteristic for the helical conformation, in agreement with the crystal structure. A marked transition in the structure of the parvalbumin was observed with the central point of 0.5 GPa (at room temperature). The amide I position shifts to a wavenumber which is between the helical and the unfolded position. We assign this change to a native-molten globule transition. It was reversible as seen from the infrared spectra.

Temperature experiments show a two-step unfolding of the protein. Above 45 °C we have molten globule structure. Complete unfolding occurs above 70 °C. The unfolding is accompanied with aggregation of the protein. The aggregation can however be prevented by relatively low pressure of 0.2 GPa.

P-091

Development of a biomembrane sensor based on reflectometry

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It has been proven in the past that reflectometric interference spectroscopy (RIfS) is a powerful measurement system for the detection of protein-protein interactions[1]. We present here the development of a reflectometric sensor which allows for the detection of membrane-protein interactions in the micromolar regime. In this study we employ two different instrumental assemblies. The first installation enables direct detection and quantification of the interaction of membrane proteins with solid supported lipid bilayers. In the second assembly the original instrument is combined with an upright fluorescence microscope. The advantages of this installation are the direct optical control of the experiment as well as a smaller sensing area. The set-up allows for the detection of interactions on lipid-patches of just several micrometers in diameter. The aim of this work is an experimental system that enables the measurement of transport processes through lipid membranes. We attempt to achieve this by covering a closed porous substrate with a lipid membrane. The first steps to reach this goal were done by spanning membranes over anodized aluminum oxide substrates.

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P-092

The incorporation of Hypericin and Emodin into artificial lipid membranes

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Hypericin (Hyp) has been studied because of its possible application in photodynamic therapy of cancer. Emodin (Emd) is used as a precursor for Hyp synthesis. The transport of Hyp and Emd through the cell membrane is of major importance for their delivery into target tumor cells. Hyp and Emd form non-fluorescence aggregates in aqueous solutions, but are dissolved in the lipid membrane in their monomer form. Black lipid membranes (BLMs) were formed by painting DPhPC onto a hole connecting two chambers of a Teflon cell. After forming the BLM Hyp or Emd was added into one of the chambers. Hyp and Emd monomers incorporated into the BLM were detected by measuring their autofluorescence signal using 532 nm and 405 nm lasers excitation, respectively. Characteristic time of the fluorescence signal increase during the incorporation of Hyp and Emd into the BLM was found to be approx. 50 and 10s, respectively. This time period has two components. First, there is a diffusion time of the studied drug molecules across the boundary layer near the BLM surface. Second, there is the time need for the incorporation of the adsorbed molecules into the BLM.

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P-093

FRET, FCCS and MD-modeling reveal unexpected homodimerization of the Fos transcription factor in live cells

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Fos and Jun, members of the activator protein 1 family of transcription factors, play a role in the regulation of a wide range of physiological processes and are also known as oncogenes. They belong to the basic-region leucine-zipper proteins binding to DNA as heterodimers. Whereas purified Jun can also form homodimers (less stable than the heterodimer), earlier in vitro studies showed that Fos was mainly monomeric. The instability of Fos homodimers was attributed to the electrostatic and hydrophilic properties of the leucin-zipper.

We have previously shown by fluorescence crosscorrelation spectroscopy that Fos and Jun formed stable complexes in HeLa cells and described their C terminal conformation using FRET and molecular dynamic modeling. Here we give evidence that Fos can also form homodimers in live cells, and estimate their K_d. FRET efficiency between Fos-CFP and Fos-YFP increased with increasing expression level and increasing acceptor-to-donor ratio up to $E \sim 4\%$. Truncation of the C termini by 165 AA brought the dyes closer together resulting in $E \sim 10\%$. FCCS corroborated stable association. MD modeling also indicated that stable homodimers could be formed. Our results introduce Fos homodimers as a new form of AP-1, possibly a new oncogenic form in Fos-over-expressing tumors.

P-094

Expression and biophysical characterization of cardiac leiomodin 2

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Initiation of actin polimerization in cells requires nucleation factors. A pointed-end-binding protein of F-actin -the leiomodin2- acts as a strong filament nucleator in muscle cells. The dynamical, structural and kinetic properties of a protein can provide important information to understand the intramolecular events underlying its function. We are interested in how does the leiomodin2 regulate the actin polimerization. Our aim is to determine the dissociation constant of the actin-leiomodin2 complex, and study a possible side-binding effect of the leiomodin2. The cardiac leiomodin2 of *Rattus norvegicus* is a 50 kDa molecular weight protein, which contains a 17 kDa N-terminal, a 18 kDa leucin reach repeat (LRR) and a 15 kDa C-terminal region. The N-term and the LRR regions are together tropomodulin homologues. We expressed the wild type the N-term+LRR the LRR+C-term and the C-term protein fragments by using a pTyB1 vector that contains an amplicillin resistance gene. The expression of the proteins was carried out with the Twin-CN (NE Bio-Labs) kit, which is a chitin-intein self-cleavage and purification system. The nucleation activity of leiomodin and the polimerization speed of actin in the presence of tropomyosin and leiomodin were studied by using pyrene-actin polimerization assay. We measured the stoichiometric, conformational and kinetic properties of the leiomodin-actin complexes with co-sedimentation assay, fluorescence spectroscopic and rapid-kinetic methods. The results showed that the rate of actin polimerization depended on the leiomodin2 concentration. The nucleator activity of leiomodin2 was ionic strength dependent. The data also confirmed that leiomodin2 is a side-binding and pointed-end binding protein of F-actin. The binding of leiomodin2 to the sides of the actin filaments was slower than to the pointed-end of the F-actin. The structure of F-actin was changed by the side-bound leiomodin2. These observations will contribute to the better understanding of the development and function of thin filaments in cardiac and other muscle tissues.

P-095

The influence of doxorubicin transferrin conjugate on the human leukemic lymphoblast cells

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Leukemias are one of the most common malignancy worldwide. There is a substantial need for new chemotherapeutic drugs effective against these diseases. Doxorubicin (DOX), used for treatment of leukemias and solid tumors is poorly efficacious when administered systemically at conventional doses. Therefore, in our study, to overcome these limitations, we used transferrin (TRF) as a drug carrier. We compared the effect of DOX and doxorubicin-transferrin conjugate (DOX-TRF) on human leukemic lymphoblasts (CCRF-CEM). The in vitro growth-inhibition test, XTT assay, indicated that DOX-TRF was more cytotoxic for leukemia cells than DOX alone.

In our researches we also evaluated the alternations of mitochondrial transmembrane potential ($\Delta\Psi_{m)}$, and production of reactive oxygen species (ROS). We monitored the $\Delta\Psi_m$ using dye JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine). The level of ROS was studied using the fluorescent probe $DCFH_2$-DA (2', 7'-dichlorodihydrofluorescein diacetate). The results demonstrate that DOX-TRF induced, decrease of mitochondrial membrane potential and significantly higher production of ROS compared with DOX treated cells. Moreover, all these results seem to be correlated with DNA fragmentation analyzed by DNA ladder. The tested processes were partially inhibited by the antioxidant, N – acetylocysteine (NAC).

The changes induced by DOX-TRF conjugate and free drug were suggest the different mechanism of action of DOX alone and conjugated with transferrin.

O-096
Time-resolved detection of protein-protein interaction
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Revealing molecular mechanism of a protein reaction has been a central issue in biophysics. For that purpose, a variety of time-resolved spectroscopic methods have been developed. However, most of them can monitor only dynamics associated with an optical transition and it has been very difficult to trace processes without optical transition. We used the pulsed laser induced transient grating (TG) method to study spectrally silent reactions of various proteins in time-domain. Here we will show studies on PixD.
PixD is a 17 kDa short protein which consists of the BLUF domain and additional short helices, and is involved in phototactic movement. The photochemical reaction studied by absorption spectroscopy revealed that this protein exhibits typical photochemistry of the BLUF proteins. The red-shifted intermediate is generated within a 100 ps. The spectrum does not change after this initial reaction, and returns back to the dark state with a time constant of 12 s at room temperature. We studied the reaction of this protein by our method and found that the protein-protein interaction is drastically changed during the reaction. The details and the biological meaning will be presented.

P-097
Molecular motions and exchange processes in human ileal bile acid-binding protein
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Human ileal bile acid-binding protein (I-BABP) plays a key role in the enterohepatic circulation of bile salts. Previously we have shown that the protein has two binding sites and tri- and dihydroxy bile salts bind with strong and moderate positive cooperativity, respectively. Positive cooperativity is thought to be related to a slow conformational change in the protein.
Our current study is directed at the structural and dynamic aspects of molecular recognition in human I-BABP using nmR spectroscopy and other biophysical techniques. As a first step in the investigation, ^{15}N relaxation nmR experiments have been employed to characterize the backbone motion in the *apo* and *holo* protein on a wide range of timescales. Our results show a moderately decreased ps-ns flexibility in the ligated protein, with most significant ordering near the portal region. In addition, the measurements indicate a slow μs-ms fluctuation at four distinct segments in the *apo* protein, a motion not observed in the doubly-ligated form at room temperature. Our studies support the hypothesis of an allosteric mechanism of binding cooperativity in human I-BABP. To shed more light on the molecular details of the binding mechanism, a site-directed mutagenesis study is in progress.

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P-098
DNA binding of newly synthesized porphyrin-tetrapeptide conjugates
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Cationic porphyrin-peptide conjugates were recently shown to enhance the delivery of peptide moiety to the close vicinity of nucleic acids but their interaction with DNA is not yet studied. We synthesized two cationic porphyrin-peptide conjugates: Tetra-peptides were linked to the tri-cationic meso-tri(4-N-methylpyridyl)-mono-(4-carboxyphenyl)porphyrin and bi-cationic meso-5,10-bis(4-N-methylpyridyl)-15,20-di-(4-carboxyphenyl)porphyrin. DNA binding of porphyrins, and their peptide conjugates was investigated with comprehensive spectroscopic methods. Evidences provided by the decomposition of absorption spectra, fluorescence decay components, fluorescence energy transfer and CD signals reveal that peptide conjugates of di- and tricationic porphyrins bind to DNA by two distinct binding modes which can be identified as intercalation and external binding. The peptide moiety does not oppose the interaction between the DNA and cationic porphyrins.
We compared the effect of complexation on structural stability of DNA and nucleoprotein complex : HeLa nucleosomes and T7 phage. UV and CD melting studies revealed that the porphyrin binding increases the melting temperature of DNA and destabilizes the DNA protein interaction in the nucleosomes but not in the T7 phage.

P-099
Nanocalorimetric investigation of nanogels binding sites
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Nanohydrogels are porous nanosized, water-swellable, cross linked macromolecular networks, made from hydrophilic polymers. They possess high water content, and offer many advantages for polymer-based drug delivery systems: i) a tunable size from tens of nanometers to micrometers, ii) a large surface area appropriate for multivalent bioconjugation, and iii) a functionalized internal network for the incorporation of different drugs in particular, or many other biomolecules in general.
Polymeric nanoparticles based on poly(*N*-isopropylacrylamide) (PNIPA) and poly(acrylic acid) (PAA) have been characterized by Isothermal Titration Calorimetry (ITC)

technique. Additionally core shell structures made by combining a superparamagnetic core with a polymeric or copolymeric shell have been also investigated. These materials have been increasingly exploited as efficient delivery vectors. The types and the number of binding sites N per nanoparticle as well as the corresponding binding constant K, and the reaction enthalpy ΔH have been obtained for several biomolecules. If the small molecules are attached by noncovalent intermolecular interactions the large biomolecules like proteins bind mainly by electrostatic forces.

P-100

Conformational and dynamical changes of AMP.PNP-bound actin

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Actin monomers generally bind ATP or ADP. During the polymerization of actin monomers into filaments the bound ATP is hydrolysed into ADP and inorganic phosphate (Pi). The biological function of the ATP hydrolysis in actin is unclear yet. Our aim was to shed light on the molecular mechanisms attributed to the hydrolysis process. To achieve these aims we substituted the actin bound ATP with a non-hydrolyzable nucleotide analogue, AMP.PNP, and monitored the local and global conformational changes in globular and filamentous actin during the nucleotide exchange. We used two biophysical methods, electron paramagnetic resonance spectroscopy (EPR) and differential scanning calorimetry (DSC).

The results show that the rotational correlation time of the actin bound spin label was increased in case of the AMP.PNP-actin monomers, indicating conformation and flexibility changes in the actin filaments. Parallel to these spectral changes the thermal transition temperature observed in the calorimetric experiments was shifted to higher temperatures in AMP.PNP-actin. We concluded that the nucleotide replacement induced both local and global conformational and flexibility changes the actin.

P-101

Detailed characterisation of Raf-1: Rock2 pathway cross-talk

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Recent conditional mutagenesis studies identified kinase independent functions of Raf-1. Among these, its interaction with the cytoskeletal-based Rho-dependent kinase Rock2 was proven to have a direct role in Ras-driven epidermal tumorigenesis. The mode of this interaction is entirely novel, since instead of phosphorylation, a direct protein-protein interaction causes the inhibition of Rock2: the negative regulatory domain of Raf can restrain the activity of Rock2 kinase domain. In cells, both kinases can exist as homodimers, but whether Rock homodimerisation and/or homo/heterodimerisation of Raf are necessary for the Raf-1:Rock2 interaction is still unknown. We found that Rock dimerisation is dispensable, whereas Raf dimerization is detrimentral for the interaction. Based on molecular modelling studies, the contribution of possible salt-bridges to the interaction was also tested. Production of soluble proteins for further co-crystallisation studies is underway in the laboratory, with the long-term aim of designing inhibitors of the interaction. Since Raf-1 is dispensable for epidermal homeostasis, targeting the Raf-1:Rock2 interaction could be a viable strategy for the molecular (co)therapy of Ras-driven epidermal tumours.

P-102

Nucleotide promiscuity of 3-phosphoglycerate kinase: implications for design of anti-HIV compounds

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The wide nucleotide specificity of 3-phosphoglycerate kinase (PGK) allows its contribution to the effective phosphorylation (activation) of nucleotide-based pro-drugs against HIV. Here the structural basis of the nucleotide-PGK interaction is characterised in comparison to other kinases, namely pyruvate kinase (PK) and creatine kinase (CK) by enzyme kinetic and structural modelling studies. The results evidenced favouring the purine *vs*. pyrimidine base containing nucleotides for PGK rather than for PK or CK. This is due to the exceptional ability of PGK in forming the hydrophobic contacts of the nucleotide rings that assures the appropriate positioning of the connected phosphate chain for catalysis. The unnatural L-configurations of the nucleotides (both purine and pyrimidine) are better accepted by PGK than either by PK or CK. Further, for the L-forms the absence of the ribose OH-groups with PGK is better tolerated for the nucleotides with purine rather than pyrimidine base. On the other hand, positioning the phosphate chain of both purines and pyrimidines with L-configuration is even more important for PGK, as deduced from the kinetic studies with various nucleotide-site mutants. These characteristics of the kinase-nucleotide interactions can provide a guideline in drug-design.

P-103

Comparison of binding ability and location of two mesoporphyrin derivatives in liposomes

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Application of porphyrins as photosensitizers (PS) is based on their light-triggered generation of reactive oxygen species (ROS) that may cause oxidative damages and ultimately kill cells. Cellular membranes are the action grounds of many sensitizers due to their amphiphilic character as well as the location of many of the targets attacked by ROS. Hence, the binding ability and location of porphyrins in liposomes as simple models of cellular membranes are of outstanding interest. Here we compare two similar mesoporphyrin (MP) derivatives, namely, MP IX dimethyl ester (MPE) and MP IX dihydrochloride (MPCl). Monocomponent small unilamellar vesicles formed of different phosphatidylcholines with incorporated MPs were investigated. We determined the binding parameters by conventional spectroscopy and the inhomogeneous distribution functions (IDFs) by fluorescence line narrowing (FLN) spectroscopy. We found that the binding ability of MPE is considerably higher than that of MPCl. IDFs show three distinct binding sites. Based on a consistent interpretation at the molecular level the "site I" is between the two lipid layers (for MPE), "site II" is deeply between the hydrocarbon chains (for both) and "site III" is between the head groups (for MPCl).

P-104

The role of the enzyme types ATP-ases in the muscle contraction

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The myofibrilla assuring muscle contraction gains energy to the slipping in mechanisms and the degree of efficiency of this process will decisively be determined by the velocity of recombination of the ATP molecule. In this there play a particular part the Na^+–K^+–ATP-ase and Mg^{++}–ATP-ase enzymes. Chemical reactions taking place in the living organism are catalyzed by enzymes, so the recombination from ADP to ATP, too. This transport process can be modelled from the energetic point of view on the basis of the general transport theorem through the following formula:

$$W = K \int_{t_1}^{t_2} \int_{x_1}^{x_2} \mathrm{grad}\, a_x \, dx \, dt.$$

From the point of view of muscle contraction it is of interest that, dependent from the type of the motions whether the length of time is very short, some seconds, or we can speak about a long lasting process. In the first case one can compare the decomposition of the ATP with the avalanche effect while in the spot. Its degree of efficiency is determined by the migration and linkage velocity of the ions. Conclusion: The degree of efficiency of the muscle contraction is determined by the quantities of the two enzymes (Na^+–K^+–ATP-ase and Mg^{++}–ATP-ase) as related to each other.

P-105

Development of new fluorescent probes for amyloid detection

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Due to a direct correlation between protein misfolding and neurodegenerative disorders, the development of small organic molecules for the detection and inhibition of fibril formation is of great importance. A set of prospective dyes, recently synthesized by a nucleophilic substitution of the bromine atom in 3-bromobenzanthrone, have been tested for their specificity to amyloid, pre-fibrillar and native amyloidogenic hen egg white lysozyme by analyzing their binding isotherms (i. e. lysozyme-induced changes of the dyes' fluorescence) in terms of Langmuir adsorption model. Based on the obtained data we concluded that the majority of new probes could be suitable alternatives for preliminary fluorescent detection of both mature and immature fibrils, showing stronger affinity for aggregated protein compared to the commercially available classical amyloid marker Thioflavin T. Moreover, these fluorophores showed very weak binding to the native protein, as judged from the recovered low association constants. Although all dyes share resembling spectral properties and structure, they are supposed to have different fibril binding sites because of the different rate of water relaxation in the immediate vicinity of each fluorophore.

P-106

Fetuin-A mediated formation and ripening of colloidal calciprotein particles

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Extracellular fluids of vertebrates are supersaturated with regard to calcium and phosphate. Hence, any tissue is prone to extensive mineral deposition unless inhibitors are present. On protein level, fetuin-A is supposed to be one of the most important factors [1]. Experiments with deletion mutants have shown that the aminoterminal domain contains a beta sheet with an ordered array of acidic residues, which mediates the attachment to basic calcium phosphates [2, 3].

The inhibition is based on the formation of nanometer-sized, spherical mineral-fetuin-A colloids, denoted as Calciprotein Particles (CPPs) [2, 4]. The initially formed CPPs show hydrodynamic radii in the range of 50 nm and are only transiently stable. After a distinct lag time, they are subject to a morphological change towards larger prolate ellipsoids with hydrodynamic radii of 100-120 nm [5].
In this context, we studied the role of fetuin-A in the formation and ripening of CPPs. On the one hand, dynamic light scattering (DLS) was used to study the influence of temperature, fetuin-A concentration and mineral ion supersaturation on the kinetics of CPP ripening [6]. On the other hand, the protein fetuin-A was investigated by means of small angle X-ray scattering (SAXS) and fluorescence correlation spectroscopy (FCS).

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O-107

Experimental physics' resources for studying biological macromolecules; deep insight into DcpS enzymatic hydrolysis of short capped mRNAs

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Degradation of the mRNA cap (mRNA 5' end) by DcpS (Decapping Scavenger) enzyme is an important process of the gene expression regulation, but little is known about its mechanism. The biological role of DcpS and its potential therapeutic applications, e.g. as a novel therapeutic target for spinal muscular atrophy, make it an interesting object for biophysical investigations. The ability of DcpS to act on various short capped mRNAs will be presented. We have examined the substrate specificity and binding affinity of the enzyme in a quantitative manner, employing experimental physics' resources, such as atomic force microscopy (AFM) and fluorescence spectroscopy for enzyme kinetics and time-synchronized-titration method (TST). Applied approach resulted in several conclusions about DcpS substrate recognition. For the first time, stereoselectivity of the hydrolysis process catalysed by DcpS will be demonstrated. Presented data clearly point out the constitutive features for designing the selective inhibitors of processes in which DcpS enzyme is involved. Detailed characteristics of the techniques introduced in biophysical characteristic of DcpS may be indispensable for other nucleotide binding proteins investigation, as well as for studies of many other kinds of macromolecules.

P-108

Antifungal activity of peach defensin involves plasma membrane permeabilization

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Plant defensins are small cysteine-rich antimicrobial peptides occurring in various plant species. They share a common three-dimensional structure, stabilized by eight disulphide-linked cysteines and composed of three antiparallel β–strands and one α–helix. Most plant defensins possess antifungal or antibacterial activity but are non-toxic to mammalian and plant cells. Plant defensins induce membrane permeabilization, resulting in Ca^{2+} uptake, K^+ efflux, alkalinization of the medium, and membrane potential changes. The gene encoding for a peach (*P. persica*) defensin PpDfn1 was expressed and purified in *E. coli*. Defensin was tested for antimicrobial activity against fungi by Sytox Green assays. Data revealed that PpDfn1 has a inhibitory effect on spore germination and acts through membrane permeabilization. Biophysical analysis demonstrated that this protein was able to interact with model and natural membranes. Binding of defensin to lipid membranes was dependent on lipid composition and surface charge. Interaction with fungal membrane lipid components leads to the insertion of peach defensin into the membrane resulting in membrane destabilization.
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P-109

Quantum Dot Multispectral Imaging Detects Cell Signaling for Prostate Cancer Progression

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Background: Immunohistochemistry (IHC) color imaging and pathological evaluation of clinical specimens have been the standard practice in pathology for cancer diagnosis and detection. IHC true color imaging suffers from the inherited complications of photobleaching and autofluorescence. It cannot detect multiple biomarkers at a time on the same specimen due to the inability to un-mix the signals. A more

dynamic detection technology is needed. We developed a multiplexed quantum dot-IHC (mQD-IHC) protocol that can detect cell signaling in cells for their epithelial to mesenchymal transition (EMT) (Xing, et al. Nat Protoc. 2 (5):1152-65, 2007). In this study we extended the application of mQD-IHC to investigate potential biomarkers associated with prostate cancer (PCa) invasiveness and lethal progression.

Objectives: To establish a mQD-IHC protocol using QD light-emitting nanoparticles 1) to detect the expression/activation of critical cell signaling proteins at the single cell level; 2) to image the plasticity and lethal progression of human PCa with specific emphasis on EMT and c-MET signaling; and 3) to examine the utility of mQD-IHC in clinical PCa specimens to determine its invasion ability and predict its metastatic capability.

Results: We analyzed the co-expression and activation of osteomimicry associated biomarkers: β2-Microlgobulin (β2-M), phosphorylated cyclic AMP responsive element binding protein (pCREB) and androgen receptor (AR) in 2,100 cells from 14 localized PCa tissue areas (Gleason 3 and 4) of 10 patients with known metastatic status. The overall median % triple positive for β2-M^+/$pCREB^+$/AR^+ cells was 51.5%. The median triple positive for the samples with metastatic potential was 61% compared with those without metastatic potential (median = 0%); p = 0.01 by a *Wilcoxon rank sum test*. The results were confirmed in 11 PCa bone metastatic specimens. We also investigated the c-MET signaling in castration-resistant human PCa model or CRPC xenografts and the clinical PCa specimens and found that the downstream signal components including pAkt and Mcl-1 were activated.

Conclusion: To validate our findings, additional clinical specimens with confirmed survival data will be analyzed and the cell-signaling-network-based mQD-IHC will be automated by Vectra Image Analysis System in a high throughput manner with the hope to predict the lethal progression of PCa *prior to* clinical manifestation of distant metastases.

P-110

Towards intrinsic binding thermodynamics

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Protein ligand binding is an important field of biopharmaceutical research. There are many techniques for quantitative determination of the ligand binding. The combination of isothermal titration calorimetry (ITC) and thermal shift assay provides a robust estimate of the binding constant.

Many binding reactions are coupled to the absorption or release of protons by the protein or the ligand, conformational changes of the protein and other processes. To correlate the structural features of binding with the energetics of binding one needs to carry out a detailed thermodynamic study of the binding reaction and to determine dependencies such as pH, buffer and temperature.

Here we present a detailed thermodynamic description of radicicol binding to human heat shock protein Hsp90 and determined proton linkage contributions to observed binding thermodynamics. We calculated the pK_a of the group responsible for proton linkage, the protonation enthalpy of this group and intrinsic thermodynamic parameters for radicicol binding. The intrinsic enthalpy of radicicol binding to Hsp90 is one of the largest enthalpies observed for any protein – ligand binding. The structural features responsible for such large binding enthalpy and very favorable intrinsic binding Gibbs free energy are discussed.

Neuronal systems and modelling

O-111

Optogenetic electrophysiology

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The combination of optical imaging methods with targeted expression of protein-based fluorescent probes enables the functional analysis of selected cell populations within intact neuronal circuitries. We previously demonstrated optogenetic monitoring of electrical activity in isolated cells, brain slices and living animals using voltage-sensitive fluorescent proteins (VSFPs) generated by fusing fluorescent proteins with a membrane-integrated voltage sensor domain. However, several properties of these voltage reporters remained suboptimal, limiting the spatiotemporal resolution of VSFP-based voltage imaging. A major limitation of VSFPs had been a reduced signal-to-noise ratio arising from intracellular aggregation and poor membrane targeting upon long-term expression *in vivo*. To address this limitation, we generated a series of enhanced genetically-encoded sensors for membrane voltage (named VSFP-Butterflies) based on a novel molecular design that combines the advantageous features of VSFP2s and VSFP3s with molecular trafficking strategies. The new sensors exhibit faster response kinetics at subthreshold membrane potentials and enhanced localization to neuronal plasma membranes after long-term expression *in vivo*, enabling the optical recording of action potentials from individual neurons in single sweeps. VSFP-Butterflies provide optical readouts of population activity such as sensory-evoked responses and neocortical slow-wave oscillations with signal amplitudes exceeding 1% $\Delta R/R_0$ in anesthetized mice. VSFP-Butterflies will empower optogenetic electrophysiology by enabling new type of experiments bridging cellular and systems neuroscience and illuminating the function of neural circuits across multiple scales.

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P-113

Biophysical visual virtual reality in retinotopic visual areas

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Previously, we have pointed out that biophoton production can be a controlled process that originates from regulated redox/radical reactions. Our biophoton experiments support the notion that various visual related phenomena such as discrete retinal noise, retinal phosphenes as well as negative afterimages are due to biophotons. We have also suggested a new model, stating that the brain is able to create biophysical pictures in retinotopic visual areas via redox regulated biophotons of synchronized neurons. According to our interpretation, visualization (imagery) is a special kind of representation i.e., visual imagery requires peculiar inherent biophysical processes. Our idea of biophysical visual virtual reality in retinotopic areas may be a possible biophysical basis of Kosslyn's reality simulation principle in the case of visual imagery. Long-term visual memories are not stored as biophysical pictures but as epigenetic codes. During visual imagery, top-down processes control the epigenetic encoded long-term visual information. Then, according to retrieved epigenetic information, synchronized retinotopic neurons generate dynamic patterns of biophotons via redox reactions that can produce biophysical pictures. We have also presented an iterative model involving a biophysical picture-representation without *homunculus* during visual imagery.

O-114

Opsin stability under varying stimulation conditions

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Opsin molecules are a burgeoning new tool for temporally precise neuronal stimulation or inhibition. Opsin properties are commonly characterized in cell culture or acute brain slice preparations using whole cell patch clamp techniques, where neuronal membrane voltage is fixed at the resting potential. However, *in vivo*, where neurons are firing action potentials, opsins are exposed to large fluctuations in membrane voltage and transmembrane ionic concentrations which can influence opsin function. In the case of implanted light delivery devices, stimulation light power varies as a function of brain tissue volume. We therefore investigated the stability of opsin properties across a variety of *in vivo*-like stimulation conditions. We find that off-kinetics of excitatory opsins vary significantly with holding membrane potential; channelrhodopsin (ChR2) slowing with depolarisation and ChIEF (ChR1/ChR2 hybrid) in contrast, accelerating. New ChR2 point mutation variants demonstrate stability across all membrane potentials. We additionally explore responses to initial and subsequent light pulses and find that ChIEF has the unique property of accelerating kinetics after the first light stimulation. Inhibitory opsins vary in sensitivity to light in a manner which correlates with their off-kinetics. Slower opsins, such as Mac (Leptosphaeria *maculans*), have higher sensitivity at low light power densities, saturating early relative to fast inhibitory opsins such as Arch (archaeorhodopsin) and NpHR (halorhodopsin). We discuss the relative merits of stability versus versatility of opsins under variable stimulation conditions.

P-115

Overexpression of NDM29 ncRNA induces neuron-like properties in neuroblastoma cells

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It has been previously shown that overexpression of NDM29 ncRNA in a SKNBE2-derived neuroblastoma (NB) cell line leads to cell differentiation, with a decrease of malignant potential. Here we use the patch-recording technique to characterize the ionic channel apparatus of NB cells expressing NDM29 at its basal level (Mock cells) or at 5.4 fold higher levels (S1 cells). The two cell lines shared very similar pools of functional K channels, but S1 cells displayed larger TTX-sensitive Na currents and were able to generate action potentials, while Mock cells were not. In addition, while Mock cells barely express functional GABA receptors, in the majority of S1 rapid application of GABA elicited a current with a EC_{50}=11.4 μM; this current was antagonized by bicuculline (10 μM) and potentiated by zaleplon (EC_{50}= 35 nm). In Mock cells, real time PCR evidenced a high level of $GABA_A$ α_3 subunit, while in S1 cells a significant expression of α_1 and α_4 was detected, whereas α_3 mRNA was down-regulated by 70%, confirming the development of functional $GABA_A$ receptors. In the same cell lines, the presence of specific markers and the secretion of specific cytokines confirmed that NDM29 expression leads to a differentiation process toward a neuron-like, rather than glial-like, phenotype.

P-116

Novel human glioma model in rat brains

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Rodent models of human cancers have been instructional in understanding the basic principles of cancer biology. It

was planned therefore to reconstitute a model of brain tumors in rats by orthotopic implantation of xenogenic transformed human cells. Among the genes with the most pronounced increasing expression in tumors we revealed *CHI3L1,* encoding the secreted CHI3L1 protein. Overexpression of *CHI3L1* resulted in increased cell proliferation, anchor-independent growth, and formation of tumors. Two main cellular signaling passways, MAPK and PI3K, were identified to be upregulated in the CHI3L1 expressing 293 cells. To obtain a novel glioma model, 293 cells, which stably expressed *CHI3L1* were stereotactically implanted in the striatum of female Wistar rats. Huge intracerebral tumors were observed in each of the rats under investigation. All tumors were surrounded by numerous GFAP-positive reactive astrocytes what is observed in tumors, initiated by high grade glioma cultured cells. Tumor formation by 293 cells stably expressing *CHI3L1* in rat brains strongly suggests that *CHI3L1* is involved in tumorigenesis. This model is going to be very useful for developing systems of oncoprotein synthesis targeted inhibition in glial tumor cells with minimal side effects on the normal cells.

O-117

Hematite particles in human brain

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Iron is important element used for chemical reaction catalysis and physiological cell functions. The reason of iron deposition is still unknown. Under conditions prevailing in human brain it is expected the formation of an amorphous or minute crystalline phase. We used light, scanning (SEM) and transmission electron microscopy (TEM), energy-dispersive microanalysis, electron diffraction and electron paramagnetic resonance (EPR) for investigation of iron deposits in globus pallidus of human brain. SEM revealed iron rich particles with Na, Si, P, S, Cl, Ca and Cu around glial cells. TEM revealed bumpy, solid particles of platy and sometimes rounded shape with the size of 2 μm to 6 μm. These ones were identified as hematite. EPR measurements showed the presence of Fe(III) and Cu(II), but little amount of Fe(II) can not be excluded. We consider low-temperature process of hematite formation in human globus pallidus in aqueous environment influenced by organic and inorganic factors. Chemical processes leading to nanoparticles formation can be associated with neurodegenerations such as Alzheimer or Parkinson disease.

O-118

Neuronal biophysics: optimised for information processing?

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Over the past 50 years our understanding of the basic biophysical mechanisms governing the spatio-temporal dynamics of neuronal membrane potentials and synaptic efficacies has significantly expanded and improved. Much research has focussed on how ionic currents contribute to the generation and propagation of action potentials, how subthreshold signals propagate along dendritic trees, how the active properties of dendrites shape the integration of incoming signals in a neuron, and how pre- and postsynaptic activities â and potential heterosynaptic effects a determine the way synaptic efficacies change on the short- and long-term. Yet, despite these advances, there have been no systematic efforts to relate the basic dynamical repertoire of neurons to the computational challenges neural circuits face, and in particular to explain systematically how the biophysical properties of neurons are adapted to process information efficiently under the constraints of noise and uncertainty in the nervous system. As an initial step in this direction, I will show how various biophysical properties of neurons, in particular short-term synaptic plasticity and dendritic non-linearities, can be seen as adaptations to resolve an important bottleneck in neuronal information processing: the loss of information entailed by the conversion of analogue membrane potentials to digital spike trains.

O-119

An analysis of new and existing opsins for scientific application

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The optogenetic toolbox has greatly expanded since the first demonstration of genetically-targeted optical manipulation of neural activity. In addition to the cation channel channelrhodopsin-2 (ChR2), the panel of excitatory opsins now includes an array of ChR2 variants with mutations in critical residues, in addition to other, related cation channels, and channel hybrids. The inhibitory opsin panel has similarly expanded beyond the first-described halorhodopsin (NpHR), a chloride pump, to include trafficking-enhanced versions of NpHR as well as the proton pumps Mac and Arch.
While the expansion of available opsins offers researchers an increasingly powerful and diverse selection of tools, it has also made it increasingly difficult to select the optimal tool for a given experiment. One cannot extract a comparison of opsins from the current literature, since studies differ across multiple variables known to contribute to opsin performance (e.g. expression method, light power density, stimulation protocols, etc.). Here, we provide the first empirical comparison of both

excitatory and inhibitory opsins under standardized conditions. Furthermore, we identify the set of parameters that describe the properties of an opsin in a way that is maximally relevant for biological application.

O-120
Subcellular compartment-specific distribution of voltage-gated ion channels
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Voltage-gated Na^+ (Nav) channels are essential for generating the output signal of nerve cells, the action potential (AP). In most nerve cells, APs are initiated in the axon initial segment (AIS). *In vitro* electrophysiological and imaging studies have demonstrated that dendritic Nav channels support active backpropagation of APs into the dendrites, but the subunit composition of these channels remained elusive. Here, I will present evidence for the somato-dendritic location of Nav channels in hippocampal pyramidal cells (PCs). Using a highly sensitive electron microscopic immunogold localization technique, we revealed the presence of the Nav1.6 subunit in PC proximal and distal dendrites, where their density is 40-fold lower than that found in AISs. A gradual decrease in Nav1.6 density along the proximo-distal axis of the dendritic tree was also detected. We have also investigated the subcellular distribution of Kv4.2 voltage-gated K^+ channel subunit and found a somato-dendritic localization. In contrast to that of Nav1.6 channels, the density of Kv4.2 first increases then decreases as a function of distance from the somata of PCs. Such subcellular compartment-specific distribution of voltage-gated ion channels increases the computational power of nerve cells.

P-121
Semantic versus syntactic: the role of the extracellular matrix on memory
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Keywords: Memory, extra cellular matrix, random walk
We expose first a biological model of memory based on one hand of the mechanical oscillations of axons during action potential and on the other hand on the changes in the extra cellular matrix composition when a mechanical strain is applied on it. Due to these changes, the stiffness of the extra cellular matrix along the most excited neurons will increase close to these neurons due to the growth of astrocytes around them and to the elastoplastic behavior of collagen. This will create preferential paths linked to a memory effect. In a second part, we expose a physical model based on random walk of the action potential on the array composed of dendrites and axons. This last model shows that repetition of the same event leads to long time memory of this event and that paradoxical sleep leads to the linking of different events put into memory.

Calcium fluxes, sparks, & waves

P-122
Fluorescence properties of quercetin in human leukemia Jurkat T-cells
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We describe the fluorescent properties of quercetin (QC) in human leukemia Jurkat T-cells. The intracellular QC spectra displayed a dominant emission maximum at 540 nm and 2 excitation maxima (380 nm, 440 nm). Our data suggest that QC elicits a specific fluorescence upon binding to intracellular proteins and that there are two dominant molecular configurations of these QC-bound proteins which are sensitive to the intracellular Ca^{2+} concentration. QC fluorescence increased after permeabilization of the cells with digitonin, suggesting that intracellular binding of QC may be down-regulated by a cytosolic factor which is lost by permeabilization. We performed also spectrofluorimetric measurements of the intracellular Ca^{2+} concentration which revealed a strong biphasic calcium release signal after cells stimulation with 50 μM QC. QC also decreased considerably the intracellular NAD(P)H level in intact cells. Taken together, these data add more evidence for the involvement of Ca^{2+} and NAD(P)H in the apoptotic pathway triggered by QC in Jurkat cells.

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P-123
Changes in myelinated axons after membrane modification
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Myelinated nerve fibres were studied with fluorescent microscopy and laser interference microscopy. Ca^{2+} redistribution during prolonged stimulation, changes in morphology and rearrangement of cytoplasmic structures were compared in normal conditions and after membrane modification by lysolecithin and methyl-β-cyclodextrin.
Lysolecithin is a detergent known to provoke demyelination, and methyl-β-cyclodextrin extracts cholesterol from membranes. Cholesterol extraction could lead to disruption of membrane caveolae-like microdomains or "rafts" and solubilisation of different proteins connected to them. Our data suggest that methyl-β-cyclodextrin and lysolecithin lead to different changes in morphology and distribution of cytoplasmic structures. The effect was different for different regions of the nerve (node of Ranvier, paranodal and internodal regions). The agents also altered the kinetics of Ca^{2+} response to stimulation in myelinated fibres.

P-124
Extracellular carbonic anhydrase contributes to the regulation of Ca2+ homeostasis and salivation in submandibular salivary gland
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The maintenance of pH in the oral cavity is important for the oral heath since even a minor drop in pH can result in dental caries and damage to the teeth. Submandibular salivary gland (SMG) is main source of fluid and electrolytes enriched saliva therefore its core for oral pH homeostasis. SMG secretion is activated by acetylcholine (Ach) in $[Ca^{2+}]_i$-dependend manner and accompanied with oral pH acidic shifts. pH shifts could be due to changes in buffering capacity that is regulated by carbonic anhydrase (CA). Despite the expression of different subtypes of CA in SMG the role of CA in the regulation of SMG function is unclear yet. We found that CA inhibition by benzolamide (BZ) decreased of fluid secretion *in vivo* extracellular Na^{2+} concentration *in situ*. The latter confirm the ability of CA to modify both primarily and final saliva secretion. We also found correlation between the secretion and Ca^{2+}-homeostasis since BZ-induced decrease of: i) total resting calcium content and stimulating effect of ACh; ii) activity of endoplasmic reticulum Ca^{2+}-ATPase; iii) mitochondrial Ca^{2+}-uptake. Thus CA contributes to saliva pH via cytosolic Ca^{2+} handling in SMG acinar cells and that this effect is mediated via cholinoreceptors.

O-125
A simple model to describe single and multichannel calcium regulation of ryanodine receptors
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In cardiac muscle, type 2 ryanodine receptor (RyR2) functioning relies on cellular Ca^{2+} levels from both background cytosolic Ca^{2+} and from neighboring open RyRs. Activation of RyR2 is brought on by calcium-induced-calcium release (CICR), but the CICR termination mechanism remains unknown. A simple physical model is developed that is able to describe the Ca^{2+} dependence of a single RyR2 and a multichannel array of RyR2. The open probability of RyR2 is defined through a two-site Hill function comprised of one activation site and one inactivation site per subunit. The diffusion equation for a steady state point source defines the Ca^{2+} concentration in the region of the RyR2 activation and inactivation sites, for which experimental Ca^{2+} dissociation constant values were used. Metropolis Monte Carlo simulations show open probabilities for one and two RyR2s consistent with electrophysiology measurements. The model shows that large RyR2 clusters exhibit a sharp transition in open probability as a function of RyR Ca^{2+} current at physiological cytosolic Ca^{2+} levels. Such a characteristic may suggest not only an activation mechanism for multiple RyR2, but also a method of CICR termination in clustered RyR2 channels.

O-126
High sensitivity of Ca2+ wave propagation to ryanodine receptor inhibition in cardiac myocytes
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In ventricular cardiac myocytes, global Ca^{2+} release from the sarcoplasmic reticulum (SR) is accomplished through the triggering of ryanodine receptors (RyR) via Ca^{2+} influx from voltage-gated Ca^{2+} channels of the cell surface. Spontaneous, propagating Ca^{2+} release events (Ca^{2+} waves) are associated with the generation of arrhythmogenic currents. These also constitute the dominant mode of global Ca^{2+} release in cells with sparse t-tubular networks, such as atrial myocytes, representing an alternate, non-triggered form of global SR Ca^{2+} release. The mechanism of propagation is however still under debate [1,2]. In this study it will be shown that partial, irreversible inhibition of RyR clusters using ruthenium red (an effectively irreversible blocker) had differential affects on Ca^{2+} sparks and waves. Modest inhibition of RyR clusters abolished Ca^{2+} wave propagation, whilst Ca^{2+} sparks were relatively unchanged.
Computer modeling of this system showed a dependence of cluster size on the affect of inhibition on Ca^{2+} wave propagation; with smaller clusters being more sensitive to irreversible inhibition; larger cluster fluxes were relatively unchanged by modest inhibition and so propagation was uninterrupted. This highlights the importance of smaller clusters in Ca^{2+} wave propagation.

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O-127
Alterations of ryanodine receptor (RyR) function and arrhythmogenic Ca^{2+} waves in cardiomyocytes
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In cardiomyocytes, spontaneous Ca^{2+} release from the SR can occur in diastole, as Ca^{2+} sparks. Sparks arise from accidental openings of a few ryanodine receptors (RyRs). However, under pathophysiological conditions, chain reactions of sparks can occur as Ca^{2+} waves. SR Ca^{2+} overload and (hyper)-phosphorylation are known to favor wave occurence. We reported that changes of the luminal SR Ca^{2+} concentrations mediated by SERCA contribute to wave propagation, by sensitizing the RyRs for cytosolic Ca^{2+}. β-Adrenergic stimulation also speeds up Ca^{2+} waves. This could be mediated via SERCA stimulation and thus SR Ca^{2+} loading. Alternatively, Ca^{2+} waves may travel faster because of RyR phosphorylation. Using a transgenic mouse lacking the RyR phosphorylation site at serine 2808 (S2808A), we obtained further insight into spreading of Ca^{2+} waves. We consistently observed acceleration of Ca^{2+} waves upon β-adrenergic stimulation in WT myocytes by 15%. However, Ca^{2+} waves in S2808A cells did not increase their velocity, even though successful SERCA stimulation was confirmed

as faster wave decay. Taken together these results indicate that SR luminal Ca^{2+} sensing and RyR phosphorylation may be important for Ca^{2+} wave propagation and the Ca^{2+} sensitivity of the RyRs. Support: SNF & Transcure.

P-128

Complementary methods in determining the chemical composition of renoureteral calculi

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Keywords: Urinary stones, chemical composition, FTIR spectroscopy, SEM-EDAX.

Renoureteral lithiasis is a disease with a relatively high incidence among the population. Getting the chemical structure of the stones is very important in medical practice, both in terms of surgical approaches for applying a minimal invasive treatment and also for the medical drug therapy administrated to the patients. Stones have been collected by endourological procedures and open surgery operations in urologic department. Preliminary estimation on the structure of the studied calculi was established by the X-ray exam (KUB and IVP) and urine lab tests. Structural investigation and calculi composition was performed through the FTIR spectrophotometer method and complementary SEM-EDAX electron microscopy. The combination of FTIR spectroscopy and SEM-EDAX allowed quantitative and qualitative evaluation of the components, the spatial distribution and the percent of major and trace elements present in a sample. The surface morphology of the samples and elemental analysis performed by SEM-EDAX confirms the presence of oxalate, carbonate and cystine. The final result has showed some non homogeneous structures and different concentrations of the chemical components of the stones.

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P-129

Effects of phosphatidyl-inositol-phosphates (PtdInsP) on calcium release events in mammalian skeletal muscle fibres

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In striated muscle Ca^{2+} release from the sarcoplasmic reticulum (SR) occurs when ryanodine receptors (Ryr-s) open either spontaneously or upon the stimulation from dihydropyridine receptors that are located in the adjacent transverse-tubular membrane and change their conformation when the cell is depolarized. Recent observations demonstrated that muscles from animal models of PtdInsP phosphatase deficiency suffer from altered Ca^{2+} homeostasis and excitation-contraction coupling, raising the possibility that PtdInsP-s could modulate voltage-activated SR Ca^{2+} release in mammalian muscle. The openings of a single or a cluster of Ryr-s can be detected as Ca^{2+} release events on images recorded from fibres loaded with fluorescent Ca^{2+} indicators. To elucidate the effects of PtdInsP-s on Ca^{2+} release events, images were recorded from skeletal muscle fibers enzymatically isolated from the *m. flexor digitorum breavis* of mice utilizing a super-fast scanning technique. A wavelet-based detection method was used to automatically identify the events on the images. Three different PtdInsP-s (PtdIns3P, PtdIns5P, and PtdIns(3,5)P) were tested. All these PtdInsP compounds decreased the frequency of spontaneous Ca^{2+} release events. Supported by the Hungarian National Science Fund (OTKA 75604), TéT.

O-130

Caffeine and depolarization alters the morphology of calcium spark in amphibian skeletal muscle

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Calcium sparks elicited by 1 mmol/L caffeine and by a depolarization to -60 mV were recorded at high time resolution on both x-y (30 frames/s) and line-scan images (65 lines/ms) on intact skeletal muscle fibers of the frog. While a typical spark appeared in one frame only, 17.3 and 26.0% of spark positions overlapped on consecutive frames following caffeine treatment or depolarization, respectively. While both caffeine and depolarization increased the frequency of sparks, as estimated from x-y images, the morphology of sparks was different under the two conditions. Both the amplitude (in $\Delta F/F_0$; 0.49 ± 0.025 vs. 0.29 ± 0.001; $n = 22426$ vs. 23714; mean $\pm$ SEM, $p < 0.05$) and the full width at half maximum (in μm; parallel with fiber axis: 2.33 ± 0.002 vs. 2.21 ± 0.005; perpendicular to fiber axis: 2.07 ± 0.003 vs. 1.88 ± 0.004) of sparks was significantly greater after caffeine treatment than on depolarized cells. These observations were confirmed on sparks identified in line-scan images. In addition, x-t images were used to analyze the time course of these events. Calcium sparks had significantly slower rising phase under both conditions as compared to the control. On the other hand, while the rate of rise of signal mass was decreased after depolarization, it increased in the presence of caffeine.

O-131
Calcium influx analysis by TIRF microscopy on myotubes from patients with RYR1 mutations linked to MH and CCD
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Prolonged depolarisation of skeletal muscle cells induces entry of extracellular calcium into muscle cells, an event referred to as excitation-coupled calcium entry. Skeletal muscle excitation-coupled calcium entry relies on the interaction between the 1,4 dihydropyridine receptor on the sarcolemma and the ryanodine receptor on the sarcoplasmic reticulum membrane. In this study we exploited TIRF microscopy to monitor with high spatial resolution excitation-coupled calcium entry (ECCE) in primary coltures of human skeletal muscle cells harbouring mutation in the RYR1 gene linked to malignant hyperthermia and central core disease. We found that excitation-coupled calcium entry is strongly enhanced in cells from patients with central core disease compared to individuals with malignant hyperthermia and controls. In addition, excitation-coupled calcium entry induces generation of reactive nitrogen species and causes the nuclear translocation of NFATc1. The activation of NFATc1 dependent genes is consistent with an increase of the IL6 secretion from primary coltures human myotubes from CCD patients and with fibre type 1 predominance of skeletal muscle of CCD patients.

Membrane lipids, microdomains & signalling

P-132
FTIR and calorimetric investigation of the effects of trehalose and multivalent cations on lipid structure
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The structure of membrane lipids is of fundamental importance for the integrity of cell and organelle membranes in living organisms. Membrane lipids are typically hydrated and their headgroup charges counter-balanced by solvated ions. Consequently, water loss can induce severe structural changes in lipid packing (lyotropic transitions) and can lead to the damage of lipid membranes even after rehydration. This can be one out of several factors that affect the viability of organisms undergoing desiccation. Many organisms, however, are resistant to even extreme water loss. Some of them synthesize trehalose which has been shown to be associated with survival of desiccation in phylogenetically diverse organisms (yeast, nematodes, brine shrimp, insect larvae, resurrection plants, and others). Here we have studied hydration sensitive transitions in model lipids to determine the effect of trehalose and electrostatics on lipid order. Hydration pulse-induced time-resolved Fourier-transform infrared (FTIR) difference spectroscopy was used to address hydration-dependent lipid structure as a function of trehalose. In combination with differential scanning calorimetry and studies of Langmuir-Blodget films we arrive at a structural and energetically consistent picture of how trehalose can affects lipidic phase behaviour and support a native lipid structure under water loss. Experiments were performed on model lipids with different headgroups and native lipids from desiccation-tolerant organisms.

P-133
Controlled self-assembly and membrane organization of lipophilic nucleosides and nucleic acids: perspectives for applications
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Lipophilic conjugates of nucleosides and nucleic acids such as DNA, RNA, and peptide nucleic acid (PNA) - combining assembly properties of amphiphiles and specific molecular recognition properties of nucleic acids - allow numerous applications in medicine and biotechnology. We recently observed self-assembly of microtubes, stable cylindrical structures with outer diameters of 300 nm and 2-3 μm and a length of 20-40 μm, from a cholesterol-modified nucleoside and phospholipids. Morphology and properties of these microtubes and functionalization with lipophilic DNA will be characterized. We also observed that lipophilic nucleic acids, PNA and DNA differing in their lipophilic moieties, partition into different lipid domains in model and biological membranes as visualized by hybridization with respective complementary fluorescently-labeled DNA strands. Upon heating, domains vanished and both lipophilic nucleic acid structures intermixed with each other. Reformation of the lipid domains by cooling led again to separation of membrane-anchored nucleic acids. By linking specific functions to complementary strands, this approach offers a reversible tool for triggering interactions among the structures and for the arrangement of reactions and signaling cascades on biomimetic surfaces.

P-134
Conformational dependent trafficking of P-glycoprotein with rafts
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P-glycoprotein (Pgp), an ABC-transporter playing a prominent role in multidrug resistance, demonstrate conformation-dependent endocytosis on the surface of 3T3-MDR1 cells. These cell surface transporters have a UIC2 conformation-sensitive-antibody-recognizable subpopulation, which is about one-third of the rest persisting long on the cell surface and perform fast internalization via rafts. We have identified that the rapid internalization is followed by quick exocytosis, in which the other subpopulation is not or only slightly involved. The exocytosis presents a cholesterol depletion dependent

intensification, in contrast to the internalization, which is inhibited by cyclodextrin treatment. This continuous recycling examined by total internal reflection (TIRF) microscopy increases the amount of the raft associated subpopulation of Pgps in the plasma membrane, and it might have a role in restoring the cholesterol content of the membrane after cholesterol depletion. Our presentation will summarize related endocytotic, exocytotic and recycling processes and that how does our data fit into our current notions regarding to the cholesterol and sphingomyelin trafficking.

P-135

Multivalent chelator lipids for targeting proteins into membrane nanodomains

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Membrane nanodomains based on phase-segregating lipid mixtures have been emerged as a key organizing principle of the plasma membrane. They have been shown to play important roles in signal transduction and membrane trafficking. We have developed lipid-like probes carrying multivalent nitrilotriacetic acid (tris-NTA) head groups for selective targeting of His-tagged proteins into liquid ordered or liquid disordered phases. In giant unilamellar vesicles strong partitioning of tris-NTA lipids into different lipid phases was observed. For a saturated tris-NTA lipid, at least 10-fold preference for the liquid ordered phase was found. In contrast, an unsaturated NTA lipid shows a comparable preference for the liquid disordered phase. Partitioning into submicroscopic membrane domains formed in solid supported membranes was confirmed by superresolution imaging. Single molecule tracking of His-tagged proteins tethered to solid supported membranes revealed clear differences in the diffusion behavior of the different NTA-lipids. By using BSA as a carrier, multivalent NTA lipids were efficiently incorporated into the plasma membrane of live cells. Based on this approach, we established versatile methods for probing and manipulating the spatiotemporal dynamics of membrane nano domains in live cells.

P-136

Biophysical investigation of IL-9R assembly and function in human T-lymphoma cells

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IL-9 is a multifunctional cytokine with pleiotropic effects on T cells. The IL-9R consists of the cytokine-specific α-subunit and the γ_c-chain shared with other cytokines, including IL-2 and -15, important regulators of T cells. We have previously shown the preassembly of the heterotrimeric IL-2 and IL-15R, as well as their participation in common superclusters with MHC glycoproteins in lipid rafts of human T lymphoma cells. Integrity of lipid rafts was shown to be important in IL-2 signaling. We could hypothesize that other members of the γ_c cytokine receptor family, such as the IL-9R complex, may also fulfill their tasks in a similar environment, maybe in the same superclusters.
Co-localization of IL-9R with lipid rafts as well as with the IL-2R/MHC superclusters was determined by CLSM. Molecular scale interactions of IL-9Rα with IL-2R and MHC molecules were determined by microscopic and flow cytometric FRET experiments. The role of lipid rafts in IL-9R signaling was assessed by following the effect of cholesterol depletion on IL-9 induced STAT phosphorylation.
Our results suggest the possibility that preassembly of the receptor complexes in common membrane microdomains with MHC glycoproteins may be a general property of γ_c cytokines in T cells.

P-137

Membrane organization of Fas transmembrane domain: Insights for clustering and apoptosis triggering

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To unravel the molecular processes leading to Fas clustering in lipid rafts, a 21-mer peptide corresponding to the single transmembrane domain of the death receptor was reconstituted into model membranes that display liquid-disordered/liquid-ordered phase coexistence, *i.e.* mimicking cells plasma membranes. Using the intrinsic fluorescence of the peptide two tryptophans residues (Trp^{176} and Trp^{189}), Fas membrane lateral organization, conformation and dynamics was studied by steady-state and time-resolved fluorescence techniques. Our results show that the Fas has preferential localization to liquid disordered membrane regions, and that it undergoes a conformational change from a bilayer inserted state in liquid-disordered membranes to an interfacial state in liquid-ordered membranes. This is a result of the strong hydrophobic mismatch between the (hydrophobic) peptide length and the hydrophobic thickness of liquid-ordered membranes. In addition, we show that ceramide, a sphingolipid intimately involved in Fas oligomerization and apoptosis triggering, does not affect Fas membrane organization. Overall, our results highlight ceramide role as an enhancer of Fas oligomerization, and unravel the protective function of Fas transmembrane domain against non-ligand induced Fas apoptosis.

P-138

Organization and dynamics of membrane-bound bovine α-lactalbumin: a fluorescence approach

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Many soluble proteins are known to interact with membranes in partially disordered states, and the mechanism and

relevance of such interactions in cellular processes are beginning to be understood. Interestingly, *apo*-bovine α-lactalbumin (BLA), a soluble protein, specifically interacts with negatively charged membranes and the membrane-bound protein exhibits a molten globule conformation. We have used the wavelength-selective fluorescence approach to monitor the molten globule conformation of BLA upon binding to negatively charged membranes as compared to zwitterionic membranes. Tryptophans in BLA exhibit differential red edge excitation shift (REES) upon binding to negatively charged and zwitterionic membranes, implying differential rates of solvent relaxation around the tryptophan residues. Our results utilizing fluorescence anisotropy, lifetime and depth analysis by the parallax approach of the tryptophans further support the differential organization and dynamics of the membrane-bound BLA forms. In addition, dipole potential measurements and dye leakage assays are being used in our ongoing experiments to explore the mechanism of BLA binding to membranes. These results assume significance in the light of antimicrobial and tumoricidal functions of α-lactalbumin.

O-139
Role of long-range effective protein-protein forces in the formation and stability of membrane protein nano-domains
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We discuss a realistic scenario, accounting for the existence of sub-micro-metric protein domains in plasma membranes. We propose that proteins embedded in bio-membranes can spontaneously self-organize into stable small clusters, due to the competition between short-range attractive and intermediate-range repulsive forces between proteins, specific to these systems. In addition, membrane domains are supposedly specialized, in order to perform a determined biological task, in the sense that they gather one or a few protein species out of the hundreds of different ones that a cell membrane may contain. By analyzing the balance between mixing entropy and protein affinities, we propose that protein sorting in distinct domains, leading to domain specialization, can be explained without appealing to pre-existing lipidic micro-phase separations, as in the lipid raft scenario. We show that the proposed scenario is compatible with known physical interactions between membrane proteins, even if thousands of different species coexist.

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P-140
Characterization of Brij 58 and 98-Resistant Membranes from Human Erythrocytes
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Lipid rafts are cholesterol and sphingolipid-enriched functional microdomains present in biomembranes. Rafts have been operationally defined as membrane fractions that are detergent insoluble at low temperature. Here we have characterized DRMs from erythrocytes treated with the nonionic detergents Brij58 and Brij98, at 4°C and 37°C, and compared them to DRMs obtained with Triton X-100 (TX100). We have also investigated the effect of cholesterol depletion in DRMs formation. Brij DRMs were enriched in cholesterol as well as TX100 DRMs. HPTLC analysis showed a very similar distribution of phosphatidylcholine-PC, phosphatidylethanolamine-PE and sphingomyelin-SM in Brij DRMs to that found in ghost membranes. SM-enriched DRMs were obtained only with TX100 while PE content was decreased in TX100 DRMs, in comparison to Brij DRMs. Immunoblot essays revealed that rafts markers (flotillin-2 and stomatin) were present in all DRMs. Contrary to TX100 DRMs, analysis of electron paramagnetic resonance spectra (with 5-doxyl stearate spin label) revealed that Brij DRMs are not in the liquid-ordered state, evincing the differential extraction of membrane lipids promoted by these detergents. Supported by FAPESP/CNPq (Brazil).

O-141
Magnetic Camemberts and Liposomes: New Tools for Structural Biology of Membrane Molecules
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Several biological membrane mimics have been built to investigate the topology of molecules in membranes. Among them ''bicelles'', *i.e.*, mixtures of long-chain and short-chain saturated phospholipids hydrated up to 98%, became very popular because they orient spontaneously in magnetic fields. Disk-shaped systems of 40–80 nm diameter and 4–5 nm thickness have been measured by electron microscopy and solid state nmR and can be oriented by magnetic fields with the disc-plane normal perpendicular to the field. We have been developing recently lipids that contain in one of their chains a biphenyl group (TBBPC) affording an orientation parallel to the magnetic field, in the absence of lanthanides. A large number of hydrophobic molecules including membrane proteins have been successfully embedded and static nmR afforded finding the orientation of protein helices in membranes; MAS nmR provided the 3D structure of peptides in bicelles. Biphenyl bicelles keep their macroscopic orientation for days outside the field, thus leading to combined nmR and

X-Rays experiments. TBBPC allows also construction of μm vesicles showing a remarkable oblate deformation in magnetic fields (anisotropy of 3-10) and opens the way to applications for structural biology or drug delivery under MRI.

O-142
Imaging membrane heterogeneities and domains by super-resolution STED nanoscopy
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Cholesterol-assisted lipid and protein interactions such as the integration into lipid nanodomains are considered to play a functional part in a whole range of membrane-associated processes, but their direct and non-invasive observation in living cells is impeded by the resolution limit of >200nm of a conventional far-field optical microscope. We report the detection of the membrane heterogeneities in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy. By combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS), we obtain new details of molecular membrane dynamics. Sphingolipids or other proteins are transiently ($\sim$ 10 ms) trapped on the nanoscale in cholesterol-mediated molecular complexes, while others diffuse freely or show a kind of hopping diffusion. The results are compared to STED experiments on model membranes, which highlight potential influences of the fluorescent tag. The novel observations shed new light on the role of lipid-protein interactions and nanodomains for membrane bioactivity.

O-143
Ca2+ controlled all-or-none like recruitment of synaptotagmin-1 C2AB to membranes
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Synaptotagmin-1 (Syt) is the major Ca2+ sensor that triggers the fast, synchronous fusion of synaptic vesicle with the presynaptic membrane upon Ca2+-mediated membrane recruitment of the cytosolic C2AB domain. The Ca2+-dependent recruitment of Syts C2AB domain to membranes has so far been investigated by ensemble assays. Here we revisited binding of wild type C2AB and different C2AB mutants of Syt to lipid membranes using a recently developed single vesicle assay. The hallmark of the single vesicle approach is that it provides unique information on heterogeneous properties that would otherwise be hidden due to ensemble averaging. We found that C2AB does not bind to all vesicles in a homogenous manner, but in an all-or-none like fashion to a fraction of the vesicles. The fraction of vesicles with bound C2AB is regulated by the amount of negatively charged lipids in the membrane as well as by [Ca2+] . This Ca2+ controlled all-or-none like recruitment of Syt to membranes provides a possible explanation for the strongly heterogeneous behavior of the *in vitro* model system for neuronal membrane fusion. Furthermore, heterogeneity in release probability among synaptic vesicles is a critical property in determining the output of a neuronal signaling event.

P-144
New insights in the transport mechanism of Ciprofloxacin revealed by fluorescence spectroscopy
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Keywords: Fluoroquinolones; Liposomes; Proteoliposomes; OmpF; Ciprofloxacin; Fluorescence; Anisotropy.
Fluoroquinolones are antibiotics that have a large spectrum of action against Gram negative and some Gram positive bacteria. The interaction between these species and liposomes has been cited as a reference in the understanding of their diffusion through phospholipid bilayer and can be quantified by the determination of partition coefficients between a hydrophobic phase (liposomes) and an aqueous solution. It is also known that some porins of the bacterial membranes are involved in transport mechanism of many fluoroquinolones. OmpF, a well characterized membrane protein characteristic of the outer membrane of Gram negative bacteria assumes the conformation of homo-trimer, whose monomers have two tryptophan residues (one located at the interface of monomers and the other at the interface lipid/protein). Thus, we proceeded to study the interaction of Ciprofloxacin, a second generation fluoroquinolone, with unilamellar liposomal vesicles and OmpF proteoliposomes of POPE/POPG, POPE/POPG/Cardiolipin and *E. coli* total. Partition coefficients (Kp's) and the association with OmpF proteoliposomes were determined by steady state fluorescence spectroscopy under physiological conditions (T=37°C; pH 7.4). The membrane mimetic systems used were characterized by DLS and fluorescence anisotropy.

P-145
CD44-ICAM-1 crosstalk alters MHCI rafts – A flow cytometric FRET and microscopic colocalization study
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Motivation is whether there exist differences in the pattern-forming capabilities of two adhesion molecules of different roles: CD44, mediating „dynamic" adhesion in cell rolling and ICAM-1, mediating „static" adhesion during the formation of immune-synapse. Homo- and hetero-associations of CD44, ICAM-1 and the MHCI is investigated on the nm- and μm-distance levels on LS174T colon carcinoma cells in two different conditions of lymphocyte homing: (1) With IFNγ and TNFα, both lymphokines up-regulating the expression level of MHCI and ICAM-1 and down-regulating that of CD44. (2) Crosslinking of CD44 and ICAM-1 representing receptor

engagement. The observations are explained by assuming the existence of a kinase cascade-level crosstalk between the CD44 and ICAM-1 molecules which manifests in characteristic complementary changes in the properties of cell surface receptor patterns. For the characterisation of cluster morphology new colocalization approaches were developed: (i) ,,number of first neighbours" distribution curves, (ii) ,,acceptor photobleaching FRET-fluorescence intensity fluctuation product" correlation diagrams, and (iii) ,,random gradient-kernel smoothing assisted decay" of Pearson-correlations, and (iv) K-function formalism.

P-147

Analyzing Janus kinases in living cells with single-color FCS using confocal and TIR-illumination

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Cytokine receptors of the hematopoietic superfamily transduce their signal through non-covalently bound Janus kinases. There are only 4 such kinases in humans (JAK1, 2, 3 and TYK2), which associate to 46 different cytokine receptor chains. Here we study the dynamics of GFP-tagged JAK1 and JAK3 in epithelial cells with fluorescence correlation spectroscopy (FCS). JAK1 and JAK3 behave differently in various aspects: In the absence of receptors, JAK1 still binds the membrane, whereas JAK3 diffuses homogeneously in the cytoplasm. We used FCS under total internal reflection illumination (TIR-FCS) and determined the membrane binding affinity of JAK1 to be 60 ± 36 nm. The association of JAK3 with the common gamma chain (γ_c) is very tight as shown by fluorescence recovery after photobleaching (FRAP). Molecular brightness analysis of single-point FCS shows that JAK1 diffuses as a monomer in the rather small cytoplasmic pool, whereas JAK3 diffuses as dimers, which undergo a defined oligomerization. The degree of oligomerization decays at higher concentrations, indicating that some unknown, saturable scaffold is involved. Characterizing the binding and mobility schemes of the Janus kinases may be important to further elucidate their specific and redundant effects in signal transduction.

P-148

Brain plasma membrane-enriched fractions contain and preserve the activity of mitochondrial membrane

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Plasma membrane (PM)-enriched fraction obtained through subcellular fractioning protocols are commonly used in studies investigating the ability of a compound to bind to a receptor. However, the presence of mitochondria membranes (MI) in the PM-enriched fraction may compromise several experimental results because MI may also contain the interest binding proteins. Aiming to analyze the subcellular fractioning quality of a standard sucrose density based protocol, we investigated (a) the Na^+K^+-ATPase (PM marker) and succinate dehydrogenase - SD (MI marker) activities; (b) the immunocontent of the adenine nucleotide translocator (ANT – MI membrane marker) in both PM- and MI-enriched fractions. Since several binding protocols may require long incubation period, we verified the quality of both fractions after 24 hours of incubation in adequate buffer. Our results show that PM- and MI-enriched fractions exhibit contamination with MI or PM, respectively. We did not observe any effect of incubation on Na^+K^+-ATPase activity and ANT content in both fractions. Surprisingly, SD activity was preserved in the PM- but not in MI-enriched fraction after incubation. These data suggest the need of more careful use of PM-enriched fraction preparation in studies involving PM proteins characterization.

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Membrane docking mode of the C2 domain of PKCε: an infrared spectroscopy study

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PKCε is activated by binding to membranes. Its C2 domain, in particular, binds to negatively charged phospholipids. We have used Fourier Transform Infrared Spectroscopy (FTIR) to determine the membrane docking mode of the C2 domain of this PKC by the calculation of the β-sandwich orientation when the domain is bound to different types of model membranes, using ATR-FTIR spectroscopy with polarized light. The vesicles lipid compositions used were: POPA, POPC/POPA (50:50), POPC/POPS/POPA (65:25:10), POPC/POPE/CL (43:36:21) and POPC/POPG/POPA (65:25:10). Results show that the interaction with membranes does not strongly affect the secondary structure of the domain and that some differences were found with respect to the extent of binding as a function of phospholipid composition. Small changes are only evident when the domain is bound to model membranes of POPC/POPA and POPC/POPG/POPA. In this case, the percentage of β-sheet of C2 domain increases if compared with the secondary structure of the domain in the absence of vesicles. With respect to the β-sandwich orientation, when the domain is bound to POPC/POPG/POPA membranes it forms an angle with the surface of the lipid bilayer bigger than that one observed when the domain interacts with vesicles of POPC/POPA and POPC/POPS/POPA.

P-151

Lipid selectivity as a determinant factor for HNP1 biological activity

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Human neutrophil peptide 1 (HNP1) is a human cationic defensin that present microbial activity against various bacteria (both Gram-positive and negative), fungi and viruses.

HNP1 is stored in the cytoplasmic azurophilic granules of neutrophils and epithelial cells. In order to elucidate the mode of action of this antimicrobial peptide (AMP), studies based on its lipid selectivity were carried out. Large unilamellar vesicles (LUV) with different lipid compositions were used as biomembranes model systems (mammal, fungal and bacterial models). Changes on the intrinsic fluorescence of the tryptophan residues present in HNP1 upon membrane binding/insertion were followed, showing that HNP1 have quite distinct preferences for mammalian and fungal membrane model systems. HNP1 showed low interaction with glucosylceramide rich membranes, but high sterol selectivity: it has a high partition for ergosterol-containing membranes (as fungal membranes) and low interaction with cholesterol-containing membranes (as in mammalian cells). These results reveal that lipid selectivity is the first step after interaction with the membrane. Further insights on the HNP1 membrane interaction process were given by fluorescence quenching measurements using acrylamide, 5-doxylstearic acid (5NS) or (16NS).

P-152

Study of supported lipid bilayers in interaction with nanoparticles by AFM

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Nanoparticles (NP) are currently used in many industrial or research applications (paints, cosmetics, drug delivery materials…). Recent papers demonstrate clearly their activity with biological membranes (nanoscale holes, membrane thinning, disruption). Different studies of the NP-membrane interaction suggest that parameters are particularly important, such as the NP size, their surface properties or their aggregation state. Composition of biological membranes being particularly complex, supported lipid bilayers (SLB) composed of a restricted number of lipids are usually used as simplified membrane model. Moreover, these two-dimensional systems are convenient for surface analysis techniques, such as atomic force microscopy (AFM), giving information on the morphology of the SLB and its mechanical properties. In this work, we study the behaviour of SLBs made of lipids representative of the membrane fluid phase (POPC) or of the raft phase (sphingomyelin). These SLBs are deposited on planar surfaces (mica or glass) previously recovered with silica beads (10 or 100 nm in diameter) in order to mimick the NP-membrane interaction. We will present in this work our first results obtained by AFM and fluorescence microscopy.

P-153

Effect of cisplatin on content of phospholipids in rat liver chromatin fraction

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It is well known that the eukaryotic nuclei are the sphere of lipids active metabolism. The investigations demonstrated the existence of numerous enzymes in nuclei which modulate the changes of nuclear lipids during different cellular processes. Although the nuclear membrane is accepted as the main place of the lipids localization, nearly 10% of nuclear lipids are discovered in chromatin fraction. The ability of chromatin phospolipids to regulate DNA replication and transcription was already demonstrated. The chromatin phospolipids seems to play an important role in cell proliferation and differentiation as well as in apoptosis. It seems also possible that chromatin phospholipids may be participated in realization of cisplatin antitumor effects.

The 24-hour in vivo effect of cisplatin on rat liver chromatin phospholipids was investigated. The phospholipids of rat liver chromatin were fractionated by microTLC technique. The quantitative estimation of fractionated phospholipids was carried out by computer program FUGIFILM Science Lab. 2001 Image Gauge V 4.0.

The alteration of total phospholipids content as well as the quantitative changes among the individual phospholipids fractions in rat liver chromatin after in vivo action of cisplatin was established. The total content of chromatin phospholipids was significantly decreased after the cisplatin action. Four from five individual phospholipids fractions were markedly changed after the drug action. Two cholin-content phospholipids, particularly phosphatidylcholine and sphingomyelin exhibit diversity in sensitivity to this drug: the increase of sphingomyelin content accompanied by quantitative decrease of phosphatidylcholine. The quantity of cardiolipin was markedly increased while the amount of phosphatidylinositol was decreased after the cisplatin treatment. The phosphatidylethanolamin content remained unchanged after the drug action.

It seems that high sensitivity of chromatin phospholipids exhibited to cisplatin action may play an important role in antitumor effects of this drug.

P-154

Membrane lipids and drug resistance in Staphylococci

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Staphylococci express numerous resistance mechanisms against common antimicrobials, including peptide components of the innate immune system which have been trumpeted as being likely candidates to replace our increasingly ineffective antibiotics. The membrane phospholipid lysylphosphatidylglycerol (L-PG) appears to play a key role in Staphylococcal drug resistance, since its absence in mutant bacteria renders them susceptible to a range of cationic antimicrobials. The current assumption about the role L-PG plays in drug resistance is that of facilitating charge neutralisation of the plasma membrane, leading to loss of affinity towards cationic moieties. We have investigated this phenomenon using a range of model membrane systems composed of both synthetic lipids and reconstituted natural lipid extracts, using such techniques as stopped-flow fluorescence, circular dichroism and neutron scattering. Our conclusions indicate that the initial assumptions about the role of L-PG in drug resistance are over-simplistic and certainly do not tell the whole story of the physical and biological properties of this fascinating moderator or membrane behaviour. Our findings show that L-PG does not inhibit

antimicrobial drug action by charge dampening, hinting at a different protective mechanism.

P-155

Modulation of α-toxin binding by membrane composition

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Although the alpha-toxin from *S. aureus* was the first pore-forming toxin identified, its mode of interaction with membranes is still not fully understood. The toxin forms heptameric pores on cellular and artificial membranes. The present hypothesis is that the initial binding to the membrane occurs with low affinity, and that an efficient oligomerisation, relying on clusters of binding sites, is the reason for the overall high affinity of the binding process. In order to separate the effects of increasing concentration of binding sites from this topological effect, we investigated the oligomer formation based on pyrene-fluorescence for a series of lipid compositions, where the fraction of toxin binding lipids (egg phospatidylcholine (ePC) or egg sphingomyelin (eSM)) was varied while their concentration remained constant. The results indicate that an increased local density of toxin binding sites occurring due to phase separation facilitates oligomer formation. Furthermore, the change in local environment (number of neighboring cholesterol molecules) upon domain formation also enhances oligomer formation.We thank the DFG (SFB 490) for financial support, S. Bhakdi and A. Valeva for production of the toxin and helpful discussions.

P-156

Quercetin effects on Laurdan emission fluorescence in the presence of cholesterol

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We explored quercetin effects on lipid bilayers containing cholesterol using a spectrofluorimetric approach. We used the fluorescent probe Laurdan which is *able to detect changes in membrane phase properties. When incorporated in lipid bilayers,* Laurdan emits from two different excited states, a non-relaxed one when the bilayer packing is tight and a relaxed state when the bilayer packing is loose. This behavior is seen in recorded spectra as a shift of maximum *emission fluorescence* from 440 nm at temperatures below lipids phase transition to 490 nm at temperatures above lipids phase transition values. Emission spectra of Laurdan were analyzed as a sum of two Gaussian bands, centered on the two emission wavelengths allowing a good evaluation of the relative presence of each population. Our results show that both Laurdan emission states are present with different shares in a wide temperature range for DMPC liposomes with cholesterol. Quercetin leads to a decrease in the phase transition temperature of liposomes, acting in the same time as a quencher on Laurdan fluorescence.
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P-157

Comparative molecular dynamics simulations of the caveolin CRAC motif in micelle and bilayer

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Caveolins are essential membrane proteins found in caveolae. The caveolin scaffolding domain of caveolin-1 includes a short sequence containing a CRAC motif (V_{94}TKYWFYR$_{101}$) at its C-terminal end. To investigate the role of this motif in the caveolin–membrane interaction at the atomic level, we performed a detailed structural and dynamics characterization of a cav-1(V94-L102) nonapeptide encompassing this motif and including the first residue of cav-1 hydrophobic domain (L102), in dodecylphosphocholine (DPC) micelles and in DMPC/DHPC bicelles, as membrane mimics. nmR data revealed that this peptide folded as an amphipathic helix located in the polar head group region. The two tyrosine side-chains, flanked by arginine and lysine residues, are situated on one face of this helix, whereas the phenylalanine and tryptophan side-chains are located on the opposite face (Le Lan C. et al., 2010, Eur. Biophys. J., 39, 307-325). To investigate the interactions between the CRAC motif and the lipids, we performed molecular dynamics simulations in two different environment: a DPC micelle and a POPC bilayer. The results obtained are in good agreement with nmR data and the comparison between both systems provided insight into the orientation of the CRAC motif at the membrane interface and into its interactions with lipids.
This work was partially supported by the strategic grant POSDRU/21/1.5/G/13798, inside POSDRU Romania 2007-2013, co-financed by European Social Found-Investing in People and by the bilateral collaboration project Brancusi Romania France, 3V/21J.

P-158

Effect of hydroxyl groups in the sphingolipid backbone on sterol/sphingomyelin interaction

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D-*ribo*-phytosphingosines are biologically significant natural sphingolipids possessing two hydroxyl groups at positions 3 and 4 in their backbone. These are found in yeasts, plants and mammalian tissues. In this study we observed bilayer properties and interaction with cholesterol of *N*-16:0 phytosphingomyelin (PhytoSM) as well as their *N*-acyl hydroxylated (2-OH, D or L conformations) chain-matched sphingomyelin. Anisotropy of diphenylhexatriene suggested that pure *N*-16:0

PhytoSM showed higher gel-fluid transition temperature (T_m, 47°C) as compared to nonhydroxylated chain matched sphingomyelin i.e. palmitoyl sphingomyelin (T_m, 40.5°C). T_m values of pure 2-OH(D) PhytoSM and 2-OH(L) PhytoSM bilayers were 42°C and 55°C respectively. Cholestatrienol (CTL) quenching suggested that PhytoSM and 2-OH(L) PhytoSM appeared to increase sterol-rich domain stability compared to 2-OH(D) PhytoSM in fluid phospholipid bilayer. DSC data suggested that PhytoSM showed good miscibility with PSM. CTL partitioning showed that sterol affinity of PhytoSM and 2-OH(L) PhytoSM bilayers was higher as compared to 2-OH(D) PhytoSM or PSM. Our study suggests that conformation and positioning of hydroxyl groups significantly affects thermotropic properties of sphingolipids and sterol interaction.

P-159

Slow relaxation following the thermal hysteresis of new bolaamphiphiles studied by SAXS/WAXS

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The polymorphism of a new series of bolaamphiphile molecules based on *N*-(12-Betainylamino-dodecane)-octyl β-D-Glucofuranosiduronamide Chloride is investigated. The length of the main bridging chain is varied in order to modify the hydrophilic/lipophilic balance. The other chemical modification was to introduce a diacetylenic unit in the middle of the bridging chain to study the influence of the $\pi - \pi$ stacking on the supramolecular organisation of these molecules. Dry bolaamphiphiles self-organize in supramolecular structures such as lamellar crystalline structure, lamellar fluid structure and lamellar gel structure. The thermal dependence of these structures, as well as the phase transition is followed by small-angle and wide-angle X-ray scattering. Once the thermal cycle is accomplished, the system remains in the kinetically stabilized undercooled high-temperature phase at temperature of 20 °C. Subsequently, the time dependence of the relaxation to the thermodynamically stable phase is followed and very slow relaxation on the order of hours or days is observed. The study of polymorphism and the stability of various phases of this new series of bolaamphiphiles is interesting for potential application in health, cosmetics or food industry, is undertaken in this work.

P-160

Interaction of alkylphospholipid liposomes with breast cancer cells

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Alkylphospholipids have shown promising results in several clinical studies and among them perifosine (OPP) is promising for breast cancer therapy. Antitumor effect was much better in estrogen receptor negative (ER-) than in estrogen receptor positive (ER+) tumors in vivo. It is believed that APL do not target DNA, but they insert in the plasma membrane and ultimately lead to cell death.
Liposomes made of OPP and different amount of cholesterol (CH) showed diminished hemolytic activity as compared to micellar OPP, but in most cases cytotoxic activity was lower. In order to find optimal liposomal composition and to understand better the difference in the response of ER+ and ER- cells the interaction OPP liposomes with ER+ and ER- cells was studied. For liposomes with high amount of CH both cell types showed slow release of the liposome entrapped spin probe into the cytoplasm. Liposmes with low amount of CH interact better with cells but the release is faster for ER- as for ER+ cells at 37°C. Experiments with nitroxide-labeled OPP (SL-OPP) liposomes suggest that the exchange of SL-OPP between liposomes and cellular membranes is fast. However, translocation of SL-OPP across the plasma membrane is slow, but seems to be faster for OPP resistent, ER+ cells as for ER- cells at 37°C.

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Estimation of a membrane pore size based on the law of conservation of mass

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The size of biomembrane pores determines which solutes or active compounds may enter the cell. Here, using a mathematical model of a lipid bilayer and the law of conservation of mass, we calculate the radius of a membrane pore created by rearranging the lipid molecules (the pore wall was formed out of the lipid heads taken from the membrane regions situated directly above and below the pore, prior its formation). Assuming a constant number of lipid molecules per bilayer (with or without the pore) and based on the literature data (60% decrease in the area per chain for a fluid-to-gel transition and a matching change of one chain volume not exceeding 4%) we have shown that the pore radius can measure up to 4.7nm (for a 7nm thick lipid bilayer) without the lipid molecules undergoing a phase transition. A further assumption of area per chain being modified as a consequence of the lipids conformational changes has resulted in an increase of the calculated radius up to 7.1nm. Finally, a comparison of the pore volume with the corresponding volume of the lipid bilayer has led to a conclusion that for the system under consideration the membrane pore can only be created with the lipids undergoing fluid-to-gel conformational changes.

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Transient STAT recruitment into submicroscopic domains at the membrane skeleton

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The type I interferon receptor is comprised of two subunits IFNAR1 and IFNAR2, which are cross-linked by their ligand.

The key signaling pathway involves tyrosine phosphorylation of signal transducers and activators of transcription (STAT1 and STAT2) by receptor-associated Janus kinases. We aim to unveil of the very early events of signal activation including ligand-induced receptor assembly and the recruitment of the cytoplasmic effector proteins STAT1 and STAT2 in living cells. To this end, we have explored the spatiotemporal dynamics of STAT recruitment at the membrane on a single molecule level. Highly transient interaction of STATs to membrane-proximal sites was detected by TIRF-microscopy, allowing for localizing and tracking individual molecules beyond the diffraction limit. Thus, we obtained a pattern of the spatio-temporal recruitment of STAT molecules to the plasma membrane revealing distinct submicroscopic structures and hotspots of STAT interaction with overlapping recruitment sites for STAT1 and STAT2. Strikingly, these STAT binding sites were independent on receptor localization and expression level. Simultaneous superresolution imaging of the cytoskeleton revealed the organization of STAT recruitment sites within the cortical actin skeleton.

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Nanoscale fluorescence correlation spectroscopy on intact living cell membranes with NSOM probes

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Characterization of molecular dynamics on living cell membranes at the nanoscale level is fundamental to unravel the mechanisms of membrane organization and-compartmentalization. We have recently demonstr ated the feasibility of fluorescence correlation spectroscopy (FCS) based on the nanometric illumination of near-field scanning optical microscopy (NSOM) probes on intact living cells [1]. NSOM-FCS was applied to study the diffusion of fluorescent lipid analogs on living CHO cells. The experiments allowed to reveal details of the diffusion hidden by larger illumination areas and associated with nanoscale membrane compartmentalization. The technique also offers the unique advantages of straightforward implementation of multiple color excitation, opening the possibility to study nanoscale molecular cross-correlation. Furthermore, the NSOM probe evanescent axial illumination allows to extend diffusion study to the membrane-proximal cytosolic region. As such, NSOM-FCS represents a novel powerful tool to characterize the details of many biological processes in which molecular diffusion plays a relevant role.

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P-164

Formation of ternary and quaternary lipid bilayers with nano/microdomains on bare and modified gold

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The growing interest in supported lipid bilayers (SLBs) on conductive substrates, such as gold, is due to the possibility of designing lipid-based biosensor interfaces with electrochemical transduction. Due to the hydrophobicity of gold it is still a challenge to deposit planar and continuous bilayers without previous surface modification. Most studies on gold concern single-phase SLBs without cholesterol or gangliosides, two vital components of biomembranes.
In this work the experimental conditions suitable for the formation of complex SLBs with phase-separation directly on gold are exploited. The mixtures DOPC/DPPC/cholesterol (4:4:2) with 0 or 10 mol % of ganglioside GM1, which should yield lipid raft-like domains according to reported phase diagrams, were studied. SLB with lipid rafts were successfully formed onto bare Au (111), although surface modification with 11-mercapto-undecanoic acid SAM stabilized the SLBs due to its charge and hydrophilicity. The different experimental conditions tested had an impact on nano/microdomains organization observed by atomic force microscopy in buffer solution. Surface characterization through the combined use of ellipsometry, cyclic voltammetry and AFM allowed to optimize the conditions for the formation of more planar and compact SLBs.

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Mechanism of amyloid formation by Aβ in raft-like membranes containing ganglioside clusters

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It is widely accepted that the conversion of the soluble, nontoxic amyloid β-protein (Aβ) monomer to aggregated toxic Aβ rich in β-sheet structures is central to the development of Alzheimer's disease. However, the mechanism of the abnormal aggregation of Aβ in vivo is not well understood. We have proposed that ganglioside clusters in lipid rafts mediate the formation of amyloid fibrils by Aβ, the toxicity and physicochemical properties of which are different from those of Aβ amyloids formed in solution [1, 2]. In this presentation, we report a detailed mechanism by which Aβ-(1-40) fibrillizes in raft-like lipid bilayers composed of GM1/cholesterol/sphingomyelin. At lower concentrations, Aβ formed an α helix-rich structure, which was cooperatively converted to a β sheet-rich structure above a threshold concentration. The structure was further changed to a seed-prone β structure at higher concentrations. The seed recruited Aβ in solution to form amyloid fibrils.

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P-167

Alterations in membrane cholesterol potentiate carbohydrate-dependent neutrophil degranulation

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At present there is a lot of controversy about lipid raft role – glycolipid- and cholesterol-rich membrane microdomains – in neutrophil signal transduction. As a large fraction of neutrophil receptors is glycosylated, this study was designed to clarify lipid rafts role in carbohydrate-dependent neutrophil degranulation. The effect of methyl-β-cyclodextrin (MβCD), a well-known lipid raft disrupting agent, on neutrophil degranulation induced by 8 plant lectins with different carbohydrate-binding specificity was examined. Lectins from Phaseolus vulgaris (PHA-E), Triticum vulgaris (WGA), Vicia sativa (VSA), Canavalia ensiformis (ConA), Caragana arborescens (CABA), Glycine hispida (SBA), Sambucus nigra (SNA) and Urtica dioica (UDA) were used for cell activation. Activity of lysozyme released from cells was assayed by measurement of the lysis rate of *Micrococus lysodeikticus*. Cholesterol extraction by MβCD resulted in potentiating of neutrophil degranulating response to VSA, SBA, UDA, SNA and PHA-E, but had no effect on WGA-, ConA- and CABA-induced degranulation. Our results demonstrate that MβCD effects on neutrophil degranulation depend on the lectin-induced signal transduction pathways and are readily resulted from recruitment of raft domains from the specific granules. The observed synergetic effect MβCD and lectin on neutrophil degranulation apparently takes place for those lectins, which receptors are associated with lipid rafts.

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The hedgehog receptor patched is involved in cholesterol transport

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Background: Sonic hedgehog (Shh) signaling plays a crucial role in growth and patterning during embryonic development, but also in stem cell maintenance and tissue regeneration in adults. Aberrant Shh pathway activation is involved in the development of many tumors, and one of the most affected Shh signaling steps found in these tumors is the regulation of the signaling receptor Smoothened by the Shh receptor Patched. In the present work, we investigated Patched activity and the mechanism by which Patched inhibits Smoothened.
Methodology / Principal Findings: Using the well-known Shh-responding cell line of mouse fibroblasts NIH 3T3, we first observed that the enhancement of the intracellular cholesterol concentration induces Smoothened enrichment in the plasma membrane, which is a crucial step for the signaling activation. We found that binding of Shh protein to its receptor Patched, which involves Patched internalization, increases the intracellular concentration of cholesterol and decreases the efflux of a fluorescent cholesterol derivative (BODIPY-Cholesterol) from these cells. Treatment of fibroblasts with cyclopamine, an antagonist of Shh signaling, inhibits Patched expression and reduces BODIPY-Cholesterol efflux. We also show that the over-expression of human Patched in the yeast *S. cerevisiae* results in a significant boost of the BODIPY-Cholesterol efflux. Furthermore, we demonstrate that purified Patched binds to cholesterol, and that the interaction of Shh with Patched inhibits the binding of Patched to cholesterol.
Conclusion / Significance: Our results suggest that Patched may contribute to the cholesterol efflux from cells, and to modulation of the intracellular cholesterol concentration. This activity is likely responsible for the inhibition of the enrichment of Smoothened in the cell plasma membrane which is an important step in the Shh pathway activation.

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SNARE-mediated membrane fusion assay based on pore-suspending membranes: observing single fusion events

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We developed a new versatile single-vesicle fusion assay to study the molecular mechanisms of membrane fusion, based on pore-suspending membranes. This system offers advantages over other model membrane systems, such as black lipid membranes (BLMs) or solid supported membranes (SSMs). Specifically, it provides a solvent-free environment with aqueous compartments that are accessible on both sides of the membrane. With this assay, we were able to observe single fusion events of large unilamellar vesicles (LUVs) with pore-suspending membranes using confocal laser scanning fluorescence microscopy. Membranes were prepared by spreading of giant unilamellar vesicles (GUVs) on highly ordered porous silicon substrates. GUVs were doped with the fluorescent dye Oregon Green DHPE, LUVs with Texas Red DHPE. Membrane fusion could be observed through Förster resonance energy transfer that occurs if the two lipid dyes come in close proximity as a result of lipid mixing. Membrane fusion was mediated using SNARE-derived artificial peptides, consisting of a coiled-coil-forming three-heptad repeat segment that is linked to a SNARE-transmembrane domain anchoring it to the lipid membrane.

Currently, a setup is developed to be able to also monitor the content release that occurs upon fusion.

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Effect of dl-alpha-tocopherol glicosidic derivative on phospholipids monolayer structure

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Phospholipid dipalmitoyl phosphatidylcholine (DPPC) and its binary mixtures with dl-α-tocopheryl β-D-glucopyranoside* (BG) in Langmuir and Langmuir–Blodgett films have been studied. Surface pressure versus mean molecular area (π–A) isotherms for Langmuir films were recorded. Langmuir-Blodgett monolayers were visualized with atomic force microscopy (AFM).

The plateau surface pressure of BG/DPPC mixtures monolayers is shifted proportionally with increasing concentration of BG. This flat region of the curve disappears for monolayers with mole fraction (MF) of BG greater than 0.2. At higher surface pressures the isotherms exhibit composed character. To obtain information about miscibility mean molecular area was analyzed. For MF$\leq$0.3 the excess area per molecule parameter, A_E, possess value in the range of measuring error what suggests either complete miscibility, or ideal phase separation of the components. For MF$>$0.3 the positive deviation of A_E is observed, indicating partial miscibility and repulsive interaction between DPPC and BG molecules.

AFM image of mixed BG/DPPC monolayer with mole fraction of BG MF=0.1 shows 10 μm circular forms of liquid condensed phase which coexist with small grains of liquid expanded phase. The measured height of pure DPPC monolayer is greater than for monolayer with MF=0.1 of BG. It may be assigned to larger tilt angle of the molecules to the normal to the surface or greater elasticity of the monolayer in the presence of BG molecules. The AFM image for BG/DPPC monolayer shows the randomly distributed precipitations on borders of the condensed phase domains. They appear in the form of narrow cone with height of 2-5 nm. Since they are seen only in BG/DPPC mixture we assume that those are aggregated or crystalline forms of BG.

During π–*A* isotherms and AFM studies of monolayer of BG/DPPC mixtures it has been shown that presence of BG molecules changes the structure of DPPC monolayer in a BG concentration dependent manner. Additionally, presence of BG in the monolayer mixture increases its stability compared to DPPC monolayer.

Acknowledgments

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P-171

Interaction of segment H_1 (NS4B_H_1) from HCV protein NS4B with model biomembranes

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Hepatitis C virus (HCV) has a great impact on public health, affecting more than 170 million people worldwide since it is the cause of liver-related diseases such as chronic hepatitis, cirrhosis and hepatocarcinoma. HCV entry into the host cell is achieved by the fusion of viral and cellular membranes, replicates its genome in a membrane associated replication complex, and morphogenesis has been suggested to take place in the endoplasmic reticulum (ER) or modified ER membranes. The variability of the HCV proteins gives the virus the ability to escape the host immune surveillance system and notably hampers the development of an efficient vaccine. HCV has a single-stranded genome which encode a polyprotein, cleaved by a combination of cellular and viral proteases to produce the mature structural proteins (core, E1, E2, and p7) and non-structural ones (NS2, NS3, NS4A, NS4B, NS5A and NS5B), the latter associated with the membrane originated from the ER in the emerging virus. The NS4B protein, a fundamental player in the HCV replicative process and the least characterized HCV protein, is a highly hydrophobic protein associated with ER membranes. It has recently been shown that the C-terminal is palmitoylated and the N-terminal region has potent polymerization activity. The expression of NS4B induces the formation of the so called membranous web, which has been postulated to be the HCV RNA replication complex. Thus, a function of NS4B might be to induce a specific membrane alteration that serves as a scaffold for the formation of the HCV replication complex and therefore has critical role in the HCV cycle. Due to the high hydrophobic nature of NS4B, a detailed structure determination of this protein is very difficult. The NS4B protein is an integral membrane protein with four or more transmembrane domains. The C-terminal region of NS4B is constituted by two α helices, H_1 (approximately from amino acid 1912 to 1924) and H_2 (approximately from amino acid 1940 to 1960), which have been studied as potential targets for inhibiting HCV replication. Previous studies from our group, based on the study of the effect of NS4B peptide libraries on model membrane integrity, have allowed us to propose the location of different segments in this protein that would be implicated in lipid-protein interaction. Additionally, the H_1 region could be an essential constituent in the interaction between protein and membrane. In this study we show that peptides derived from the C-terminal domain of NS4B protein of HCV are able to interact with high affinity to biomembranes, significantly destabilizing them and affecting their biophysical properties. There were also differences in the interaction of the peptide depending on the lipid composition of the membranes studied. We have also applied fluorescence spectroscopy, infrared spectroscopy and differential scanning calorimetry which have given as a detailed biophysical study of the interaction of the peptide with model biomembranes.

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P-172

Theoretical and computational models for explaining the enhancement of the fusion rate of charged membranes in presence of water soluble polymer

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A semi-quantitative theory describing the adhesion kinetics between soft objects as living cells or vesicles was developed. The nucleation-like mechanism has been described in the framework of a non-equilibrium Fokker-Planck approach accounting for the adhesion patch growth and dissolution (A. Raudino, M. Pannuzzo, J. Chem. Phys. **132**, 045103 (2010)). A well known puzzling effect is the dramatic enhancement of the adhesion/fusion rate of lipid membranes by water-soluble polymers that do not specifically interact with the membrane surface.

We extend the previous approach by Molecular Dynamics simulations in the framework of a coarse-grained picture of the system (lipid+polymer+ions embedded in an explicit water medium) in order to test and support our previous analytical results.

Simulations show that the osmotic pressure due to the polymer exclusion from the inter-membrane spacing is partially balanced by an electrostatic pressure. However, we also evidenced an interesting coupling between osmotic forces and electrostatic effects. Indeed, when charged membranes are considered, polymers of low dielectric permittivity are partially excluded from the inter-membrane space because of the increased local salt concentration. The increased salt concentration means also a larger density of divalent ions which form a bridge at the contact region (stronger adhesion). The overall effect is a smaller membrane repulsion. This effect disappears when neutral membranes are considered.

The model could explain the fusion kinetics between lipid vesicles, provided the short-range adhesion transition is the rate-limiting step to the whole fusion process.

P-173

The role of ceramide acyl chain length and unsaturation on membrane structure

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Ceramide fatty acid composition selectively regulates distinct cell processes by a yet unknown mechanism. However, evidence suggests that biophysical processes are important in the activation of signalling pathways. Indeed, ceramide strongly affects membrane order, induces gel/fluid phase separation and forms highly-ordered gel domains. The impact of ceramide N-acyl chain in the biophysical properties of a fluid membrane was studied in POPC membranes mixed with distinct ceramides. Our results show that: i) saturated ceramide has a stronger impact on the fluid membrane, increasing its order and promoting gel/fluid phase separation, while their unsaturated counterparts have a lower (C24:1 ceramide) or no (C18:1 ceramide) ability to form gel domains at physiological temperature, ii) differences between distinct saturated species are smaller and are related mainly to domain morphology, and iii) very long chain ceramide induces the formation of tubular structures probably associated with interdigitation. These results suggest that generation of different ceramide species in cell membranes has a distinct biophysical impact with acyl chain saturation dictating membrane lateral organization, and chain asymmetry governing interdigitation and membrane morphology.

P-174

Smoothing effect of cholesterol on a phosphatydylocholine bilayer. A molecular simulation study

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Extra high content of cholesterol (Chol) in fiber-cell membranes in the eye lens leads to Chol saturation and formation of cholesterol bilayer domains (CBDs). It is hypothesized that high enrichment in cholesterol helps to maintain lens transparency and protect against cataractogenesis. In model studies, the CBD is formed in a phospholipid bilayer when cholesterol content exceeding the cholesterol solubility threshold, thus, the CBD is surrounded by phospholipid bilayer saturated with cholesterol. In the present study, we carried out molecular dynamics (MD) simulation of two bilayers: a palmitoyloleoylphosphatidylcholine (POPC) bilayer (reference) and a 1:1 POPC-Chol bilayer, to investigate the smoothing effect of the saturating amount of cholesterol on the bilayer. To our knowledge, this effect has not been studied so far so this study is certainly providing new results. Our results indicate that saturation with cholesterol significantly narrows the distribution of vertical positions of the center-of-mass of the POPC molecules and the POPC atoms in the bilayer and smoothes the bilayer surface. We hypothesize that this smoothing effect decreases light-scattering and helps to maintain lens transparency.

P-175

Biophysical characterisation of the *Staphylococcus aureus* membrane: a neutron diffraction study

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The phospholipid content of *Staphylococcus aureus* membranes displays a high degree of variability (1-3). The major phospholipids found in *S. aureus* are phosphatidylglycerol

(PG), cardiolipin (CL) and lysylphosphatidylglycerol (LPG), (1) the concentrations of which are environment dependent and see fluctuations on exposure to high concentrations of positively charged moieties (4). Up regulation of LPG has a suspected role in neutralisation of the plasma membrane in response to cationic threats. Studies have been conducted to probe biomimetic models of this theory however our focus is to look at atomic details of membrane extracts in the presence of magaininF5W. *S. aureus* 476 lipid extracts from cells grown at pH 5.5 and 7.0, studies by neutron diffraction with and without peptide at two contrasts. D-spacings were assessed by Vogt area fitting and Bragg's law. Bilayer separation at low pH was ~1-2 Å less than pH 7.0. With peptide, bilayer separations of pH 5.5 and 7.0 extracts were reduced by ~2 Å and ~4 Å, respectively. Reduced PG content of low pH extracts is suggested to reduce D-spacing, however presence of peptide further reduces this, possibly by an anion neutralisation effect. Abnormal D-spacing on increased humidity may be due to the breakdown of LPG.

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Chlorohydrins induce oxidative stress, phosphorylation of p38 kinase and apoptosis of HUVEC-ST cells

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Activation of neutrophils releasing HOCl and apoptosis of vein endothelial cells are the events documented to occur in the course of atherosclerosis. As lipid chlorohydrins, which are the key products of the reaction between HOCl and unsaturated fatty acid residues, were found in atherosclerotic plaques, we decided to check their biological activity in the context of their ability to act as the mediators of HOCl-induced oxidative stress and apoptosis in the culture of immortalized human umbilical vein endothelial cells (HUVEC-ST). The concentration of reactive oxygen species was found to be elevated after 1 h cell incubation with phospahtidylcholine chlorohydrins. This effect was at least partially caused by the leakage of superoxide anion from mitochondria and followed by depletion of GSH and total thiols. The significant decrease of antioxidant capacity of cell extracts was also observed. The intracellular red-ox imbalance was accompanied by the increase of the ratio between phosphorylated and dephosphorylated forms of p38 MAP kinase. After longer incubation a significant number of apoptotic cells appeared. Summing up, phosphatidylcholine chlorohydrins may be regarded as signaling molecules, able to initiate signalling pathways by induction of oxidative stress.

P-179

Influence of avidin-coated surface for GUV immobilization on lipid domain size and distribution.

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Giant unilamellar vesicles (GUVs) are a valuable tool in the study of lateral distribution of biological membrane components. GUV dimensions are comparable to typical cell plasma membranes and lipid phase separation can be observed through fluorescence microscopy. GUV studies frequently require immobilization of the vesicles, and several methods are available for that effect. One of the most common methodologies for vesicle immobilization is the use of avidin/streptavidin coated surfaces and biotin labeled lipids at very low concentration in the vesicles. Here, we analyze the effect of using this methodology on lipid domain distribution for different lipid compositions. We show that as a result of non-homogeneous distribution of biotin labeled lipids between liquid disordered, liquid ordered and gel phases, distribution of lipid domains inside GUVs can be dramatically affected.

P-180

Monitoring membrane permeability: development of a SICM approach

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Scanning ion conductance microscopy (SICM) utilises a nanopipette containing an electrode as a probe for surface investigations with resolutions of 1/3 of the inner pipette diameter. Experiments are conducted under physiological conditions, *in situ* and without mechanical contact of probe and sample. Hence, SICM serves as a well-suited technique for the investigation of soft objects such as cells or artificial lipid membranes.
Using pore-suspending membranes (PSM) as a model system, interactions of melittin as an example for cell penetrating peptides (CPPs) and lipid membranes are investigated by means of SICM. Formation of a range of solvent free PSM from lipid vesicles has been achieved as confirmed by means of fluorescence microscopy and SICM. Application of melittin results in rupturing of the lipid bilayer.
Putative insights gained from this assay are critical concentrations of membrane permeabilising CCPs and answers to mechanistic questions, e.g. whether CCPs translocate only or form pores within the lipid bilayer.

P-181
Min-waves on confined artificial membranes
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Positioning of the Z-Ring in Escherichia Coli prior to cell division is regulated by intracellular pole-to-pole oscillation and membrane binding of Min proteins, allowing assembly of FtsZ filaments only at the center plane of the cell. In order to investigate the influence of membrane geometry on the dynamic behavior of membrane binding of Min proteins, we combined concepts of synthetic biology and microfabrication technology. Glass slides were patterned by a gold coating with microscopic windows of different geometries, and supported lipid bilayers (SLB) were formed on these microstructures. On SLBs, Min-proteins organize into parallel waves. Confinement of the artificial membranes determined the direction of propagation.Min-waves could be guided along curved membrane stripes, in circles and even along slalom-geometries. In elongated membrane structures, the protein waves always propagate along the longest axis. Coupling of protein waves across spatially separated membrane patches was observed, dependent on gap size and viscosity of the aqueous media above the bilayer. This indicates the existence of an inhomogeneous and dynamic protein gradient above the membrane.

O-182
Minimal systems for membrane associated cellular processes
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The strive for identifying minimal biological systems, particularly of subcellular structures or modules, has in the past years been very successful, and crucial in vitro experiments with reduced complexity can nowadays be performed, e.g., on reconstituted cytoskeleton and membrane systems. In this overview talk, I will first discuss the virtues of minimal membrane systems, such as GUVs and supported membranes, in quantitatively understand protein-lipid interactions, in particular lipid domain formation and its relevance on protein function. Membrane transformations, such as vesicle fusion and fission, but also vesicle splitting, can be reconstituted in these simple subsystems, due to the inherent physical properties of selfassembled lipids, and it is compelling question how simple a protein machinery may be that is still able to regulate these transformations. As an exciting example of the power of minimal systems, I show how the interplay between a membrane and only two antagonistic proteins from the bacterial cell division machinery can result in emergence of protein self-organization and pattern formation, and discuss the possibility of reconstituting a minimal divisome.

P-183
Quantitative microscopic analysis reveals cell confluence regulated divergence of PDGFR-initiated signaling pathways with logically streamlined cellular outputs
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Platelet derived growth factor receptors (PDGFR) play an important role in proliferation and survival of tumor cells. PDGF-BB stimulation caused a redistribution of PDGF receptors towards GM1 rich domains, which was more prominent in confluent monolayers. PDGF-BB stimulation significantly increased relative receptor phosphorylation of the Ras / MAPK pathway specific Tyr716 residues and the PI3-kinase / Akt pathway specific Tyr751 residues in non-confluent cultures. Tyr771 residues that serve as adaptors for Ras-GAP which inactivates the MAPK pathway and Tyr1021 residues feeding into the PLC-gamma / CaMK-PKC pathway were the docking sites significantly hyperphosphorylation following ligand stimulation in confluent cells. We found that p-Akt facilitated cell survival and pMAPK dependent proliferation is more activated in dispersed cells, while phospholipase C-gamma mediated calcium release and PKC-dependent RhoA activation are the prominent output features PDGF stimulus achieves in confluent cultures. These observations suggest that the same stimulus is able to promote distinctly relevant signaling outputs, namely, cell division and survival in sparse cultures and inhibition of proliferation joined with promotion of migration in confluent monolayers that appear contact inhibited.

P-184
A thermodynamic approach to phase coexistence in ternary cholesterol-phospholipid mixtures
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We present a simple and predictive model for describing the phase stability of ternary cholesterol-phospholipid mixtures. Assuming that competition between the liquid and gel organizations of the phospholipids is the main driving force behind lipid segregation, we derive a phenomenological Gibbs free-energy of mixing, based on the calorimetric properties of the lipids main transition. Gibbs phase diagrams are numerically obtained that reproduces the most important experimental features of DPPC-DOPC-Chol membranes, such as regions of triple coexistence and liquid ordered - liquid disordered segregation. Based on this approach, we present a scenario for the evolution of the phase diagram with temperature. Results for other phospholipid species, such as POPC or PSM will also be presented.
J. Wolff, C. M. Marques, and F. Thalmann Phys. Rev. Lett. **106**, 128104 (2011)

P-185

Membrane potential influences mobility, interactions and signaling of interleukin-2 and -15 receptors in T cells

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Interleukin-2 and -15 receptors play a central role in the activation, survival and death of T lymphocytes. They form supramolecular clusters with MHC I and II glycoproteins in T cells. In damaged or inflamed tissues the extracellular K+ concentration increases, which can depolarize the membrane. The common signaling beta and gamma chains of IL-2/15R are phosphorylated upon cytokine binding and get a permanent dipole moment, thus their conformation, interactions, mobility and activity may be sensitive to the membrane potential. We induced depolarization on FT 7.10 T lymphoma cells by increasing the ec. K+ level or by blocking Kv 1.3 voltage gated K+ channels with margatoxin. FCS measuremens showed that the lateral mobility of Fab-labeled IL-2/15R and MHC I and II decreased upon depolarization, while that of GPI-linked CD48 did not change. FRET efficiency measured between some elements of the IL-receptor/MHC cluster increased, which may reflect an increase of cluster size. IL-2-induced receptor activity, as monitored by measuring STAT5-phosphorylation, increased upon depolarization, whereas IL-15 induced phosphorylation did not change. Our results may reveal a novel regulatory mechanism of receptor function by the membrane potential.

P-186

MHC I organizes protein clusters and inhibits IL-2/IL-15 signaling in human T cells

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Major histocompatibility class I and II glycoproteins and interleukin-2 and -15 receptors form supramolecular clusters in lipid rafts of FT7.10 T lymphoma cells. IL-2 and IL-15 cytokines play an important role in T cell activation and immunological memory, whereas MHCs are known for the role in antigen presentation.
We applied RNAi to silence the expression of MHC I in order to study its possible role in receptor assembly and function. FRET data indicated that the association of IL-2R and IL-15R with MHC I as well as between IL-2R and IL-15R weakened. FCS indicated an increase of receptor mobility also suggesting the partial disassembly of the clusters. MHC I gene silencing lead to a remarkable increase of IL-2/IL-15 induced phosphorylation of STAT5 transcription factors. In search for the molecular background of this inhibition of signaling by MHC I we checked IL-2 binding and the formation of the receptor complex (IL-2R alpha - IL-2R beta association), but we did not find a difference as compared to the control.
Our results suggest that MHC I plays an organizing role in maintaining supramolecular receptor clusters and inhibits IL-2R signaling, revealing a nonclassic new function of MHC I beyond its classical role in antigen presentation.

P-187

Monitoring Interleukin-4 receptor activation: from the cell surface into endosomes

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Interleukin-4 (IL-4) is an important cytokine involved in adaptive immunity. IL-4 binds with high affinity the single-pass transmembrane receptor IL-4Rα. The occupied complex, IL-4/IL-4Rα then engages either IL-2Rγ or IL-13Rα1, to form an activated type I or II receptor, respectively. This formation of heterodimers is believed to trigger cross-activation of intracellular Janus kinases. Here we follow a fluorescently labeled ligand through various stages of receptor activation in HEK293T: Using fluorescence correlation spectroscopy (FCS), we see that the receptor chains diffuse as monomers within the plasma membrane. Using dual-color FCCS provides direct evidence for ligand induced co-diffusion of occupied IL-4Rα and IL-13Rα1. In contrast, type I complexes containing IL-2Rγ could not be observed. However, ectopic expression of GFP-tagged IL-2Rγ/JAK3 induced stable fluorescent speckles in or close to the plasma membrane. We identified these structures as early sorting endosomes by colocalization of surface markers like EEA1 and Rab GTPases. The IL-4Rα chain is continuously trafficking into these compartments. These observations suggest that the formation of a type I IL-4R heterodimer may require internalization and that early endosomes serve as a platform for IL-4 signaling.

O-188

Spot variable Fluorescence Correlation Spectroscopy reveals fast scouting of K-Ras at the plasma membrane of living cells

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Among the membrane associated proteins, the Ras family, which is lipid-anchored G protein, plays a key role in a large range of physiological processes and, more importantly, is deregulated in a large variety of cancer. In this context, plasma membrane heterogeneity appears as a central concept since it ultimately tunes the specification and regulation of Ras-dependent signaling processes.

Therefore, to investigate the dynamic and complex membrane lateral organization in living cells, we have developed an original approach based on molecule diffusion measurements performed by fluorescence correlation spectroscopy at different spatial scales (spot variable FCS, svFCS) (**1**). We have shown in a variety of cell types that lipid-based nanodomains are instrumental for cell membrane compartmentalization. We have also observed that these-nanodomains are critically involved in the activation of signaling pathways and are essential for physiological responses (2-3).

More recently, weextend the application of svFCS to characterizethe dynamics of K-Rasproteinat the plasma membrane. As major result, we demonstrated that the rate of K-Ras association/dissociation from the membrane is fast but vary as a functional of the activation state of the molecule as well as of specific intracellular protein interactions.

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Aggregated proteins

P-190

Insights into the molecular mechanisms of Hsp70-mediated inhibition of amyloid formation

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Molecular chaperones have been recognised as key players in the avoidance of misfolding and amyloid fibril formation. In particular, recent evidences demonstrate that loss of chaperone heat-shock protein 70 kDa (Hsp70) activity is strictly related with the formation of intra-neuronal inclusions of alpha-synuclein (AS), one of the main hallmarks of Parkinson's disease.

Human Hsp70 is composed of two functional domains; the 44-kDa N-terminal nucleotide-binding domain (NBD), with ATPase activity, and the 30-kDa C-terminal substrate-binding domain (SBD). With the aim of gaining insight into Hsp70 substrate recognition process of AS in relation with the Hsp-70 mediated inhibitory effect on AS aggregation, we have characterized both processes in detail using full-length Hsp70 and several truncated variants and by means of a wide range of biophysical techniques, including nmR.

We have so demonstrated that an helical lid sub-domain in the SBD is essential for monomeric AS binding, but not for the anti-aggregation activity of the chaperone, suggesting that Hsp70 is able to interact with pre-fibrillar oligomeric species formed during AS aggregation and that, then, the mechanism of binding for these species is different from that of the monomeric protein.

P-191

Characterizing the amyloidogenic state of the acylphosphatase from *Sulfolobus solfataricus*

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Aggregation of the acylphosphatase from *Sulfolobus solfataricus* (Sso AcP) into amyloid-like protofibrils is induced by the establishment of an intermolecular interaction between a 11-residue unfolded segment at the N-terminus and the globular unit of another molecule. We have used data from hydrogen/deuterium exchange experiments, intermolecular paramagnetic relaxation enhancements and

isothermal titration calorimetry measurements on an aggregation-resistant Sso AcP variant lacking the 11-residue N-terminus to characterize the initial steps of the aggregation reaction. Under solution conditions that favour aggregation of the wild-type protein, the truncated protein was found to interact with a peptide corresponding to the N-terminal residues of the full length protein. This interaction involves the fourth strand of the main β-sheet structure of the protein and the loop following this region and induces a slight decrease in protein flexibility. We suggest that the amyloidogenic state populated by Sso AcP prior to aggregation does not present local unfolding but is characterized by increased dynamics throughout the sequence that allow the protein to establish new interactions, leading to the aggregation reaction.

O-192

Amyloid-like aggregates alter the membrane mobility of GM1 gangliosides

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Neuronal dysfunction in neurodegenerative pathologies such as Alzheimer's disease is currently attributed to the interaction of amyloid aggregates with the plasma membrane. Amongst the variety of toxic mechanisms proposed, one involves the binding of amyloid species to GM1 gangliosides. GM1 takes part into the formation of membrane rafts, and exerts antineurotoxic, neuroprotective, and neurorestorative effects on various central neurotransmitter systems. In this study, we investigated the effects of amyloid-like aggregates formed by the highly amyloidogenic structural motif of the yeast prion Sup35 (Sup35NM) on the mobility of GM1 on the plasmamembrane of living cells.
Preformed Sup35NM aggregates were incubated with cells and GM1 molecules were subsequently labeled with biotinylated CTX-B and streptavidin quantum dots (QDs). Single QDs bound to GM1 were then tracked. The mobility of GM1 was found to decrease dramatically in the presence of Sup35NM aggregates, switching from Brownian to mainly confined motion.
The considerable interference of amyloid-like aggregates with the lateral diffusion of GM1 might imply a consequent loss of function of GM1, thus contributing to explain the toxic mechanism ascribed to this particular interaction.

P-193

Insights into the early stages of fibrillogenesis of insulin using mass spectrometry

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Insulin is a vital hormone in metabolic processes as it regulates the glucose levels in the body. Insulin is stored in the β cells of the pancreas as a hexamer, however its biologically active form is the monomer. The formation of fibrillar aggregates of insulin rarely occurs in the body; however localised amyloidosis at the site of injection for diabetes patients and aggregation of pharmaceutical insulin stocks present problems.
In the current study oligomers formed early in the process of fibril assembly *in vitro* are observed by mass spectrometry (MS). MS is the only technique which allows early species to be characterised as it can identify different oligomeric orders by mass to charge ratio and show protein abundance and aggregation propensity.
on mobility MS is used to examine rotationally averaged collision cross sections of oligomers in the aggregating solution. A wide array of oligomers is observed and the stability of specific species is remarked. The presence of multiple conformations for the highly charged oligomers is particularly noted and their assignment confirmed using Fourier Transform Ion Cyclotron Resonance MS and collision induced dissociation. Molecular modelling has been used to further explore the conformational space the oligomers inhabit.

P-194

Viscometric detection of conformational transitions and aggregation of polylysine

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Amyloid fibrils consisting of different proteins have been recognized as an accompanying feature of several neurodegenerative diseases. Many proteins without known connection to any diseases have been found to form amyloid fibrils in vitro, leading to suggestion that the ability to form fibrils is the inherent property of polypeptide chain. The observed common character of protein amyloid formation enables to seek further clues of fibrillation mechanism by studying generalized sequenceless polypeptide models, e.g. polylysine. We have studied conformational transitions of polylysine, with different chain length at various pH, ionic strength and temperature by means of novel approach - viscometric method. This polypeptide undergoes alfa-helix to beta-sheet transition and forms amyloid fibrils in special conditions. Temperature induced α-helix to β-sheet transitions occurs at pH interval form 10 to 11.8 and with increasing chain length is slightly shifted to the lower pH. We have found narrow pH interval, in which the thermal transition is fully reversible, suggesting the high sensitivity of polypeptide conformation on subtle changes in charge on its side chains.

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P-195
Flow-induced polymorphism of protein aggregates in micro channels
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Understanding the mechanisms of the conversion from the native state of a protein to the amyloidal state represents a fundamental step in improving the purification, storage and delivery of protein-based drugs and it is also of great relevance for developing strategies to prevent *in vivo* protein aggregation. Amyloid fibrils have a structural arrangement of cross β-sheet but they can also experience different packing into three dimensional superstructures, i.e. polymorphism. It is well known that, among others, both the geometric confinement of the molecules and shear forces can affect the final morphology of the aggregates. Importantly, due to the complexity and crowding of the cellular region, such parameters also play a crucial role in *in vivo* processes. We present an experimental approach to study *in vitro* amyloid aggregation in a controlled and uniform shear force field and within microscale environments. In particular we focus on the effect of these two parameters on the formation of spherical aggregates, known as spherulites. Using micro channels of different cross-sections from 5 to1000 μm x 12 μm and flow rates in the range of hundreds of μl/min, the number and diameter of spherulites within the channels have been characterized using crossed polarizers optical microscopy.

P-196
Inhibition of insulin amyloid fibrillization by albumin magnetic fluid
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Insulin amyloid aggregation causes serious problems for patient with insulin dependent diabetes undergoing long-term treatment by injection, in production and storage of this drug and in application of insulin pumps. Recent studies indicate that protein amyloid aggregation causes the cell impairment and death; however, the prevention of amyloid aggregation is beneficial. We have investigated ability of albumin magnetic fluid (AMF) to inhibit insulin amyloid aggregation by spectroscopic and microscopic techniques. Albumin magnetic fluid consists of magnetic Fe_3O_4 nanoparticles sterically stabilized by sodium oleate and functionalized with bovine serum albumin (BSA) at various weight ratios BSA/Fe_3O_4. We have found the positive correlation between inhibiting activity of AFM and nanoparticle diameter and zeta potential. The ability of AMF to inhibit formation of amyloid fibrils exhibits concentration dependence with IC_{50} values comparable to insulin concentration. The observed features make AMF of potential interest as agents effective in the solving of problems associated with insulin amyloid aggregation.

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P-197
Beta-sheet Structure Formation at the Air-Water Interface: Triggers, Transitions and Orientations
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Amyloid formation of peptides causes diseases like Alzheimer's and Parkinson's disease. However, the conditions for the onset of the neurotoxic beta-sheet formation are poorly understood.
We focus on aggregation triggers and their interplay: Interactions with hydrophobic-hydrophilic interfaces, orientation of peptides in 2D, metal ion complexation and lipid layers. The tailor-made model peptides exhibit defined secondary structure propensities and metal ion binding sites.
The interactions of the peptide with the air-water interface and with metal ions are studied using surface sensitive methods connected to film balance measurements. X-ray diffraction, X-ray reflection, infrared reflection-absorption spectroscopy and total reflection X-ray fluorescence were applied to reveal the layer structure, peptide conformations and metal ion binding at the interface.
We found that amyloid formation in 2D is dominated by the hydrophobic-hydrophilic interface and not comparable to the bulk behaviour. The interface can enhance or inhibit beta-sheet formation. The effect of metal ion complexation depends on the arrangement of the binding sites in the peptide and the preferred metal complexation geometry. The two triggers interface and metal ion complexation, can oppose each other.

P-198
Effect of ApoE isoform and lipidation status on proteolytic clearance of the Amyloid-beta peptide
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Alzheimer's disease (AD) is the most common type of dementia in the elderly. The most important genetic risk factor identified for AD is the isoform, ε2, ε3 or ε4, of apolipoprotein E (ApoE), a lipid-carrying protein. One hallmark of

AD is the accumulation of amyloid-beta peptide (Aβ) in the brain which is thought to result from an imbalance between the production of Aβ and its clearance. Previous studies report an important role for ApoE in Aβ degradation. We sought to determine the effect of ApoE isoform and lipidation status on the degradation of soluble Aβ by proteinases such as insulin-degrading enzyme and neprilysin. In this study an *in vitro* Aβ clearance assay based on the competition between Aβ and a fluorogenic peptide substrate is developed to quantify Aβ degradation. To elucidate the proteolytic clearance mechanism, the fragments resulting from cleavage are identified by mass spectrometry and further analyzed to identify the interacting stretch of the Aβ sequence with the different ApoE isoforms. The results suggest that ApoE influences the rate of Aβ degradation.

P-199

The role of protein context of polyglutamine in misfolding and aggregation: a combined biophysical and computational approach

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Polyglutamine (polyQ) diseases are a class of neurological amyloid pathologies triggered by an aberrant aggregation of proteins bearing an expanded polyQ stretch. Proteins involved in polyQ diseases do not share any functional and structural similarity besides the polyQ tract which has been accounted as the causative agent of the pathology. Nevertheless, it has been demonstrated that the aminoacidic context in which polyQ is inserted can greatly influence protein aggregation and, consequently, cytotoxicity. In this view, a deep knowledge of the structural and dynamic features of polyQ flanking sequences and their interplay within the misfolding and aggregation events are crucial to understand the molecular mechanisms leading to amyloid formation. In the present contribution, we used the multidomain polyQ protein ataxin-3 as a model for polyQ context studies, integrating computational approaches, as atomistic molecular dynamics simulations, and experimental data derived from fluorescence, FT-IR and CD spectroscopies, ESI-MS and SEC. Besides the well-known role of the N-term structured Josephine domain (JD) in the protein aggregation pathway, our studies highlighted a crucial role for the disordered tract between JD and polyQ, as well as for the C-terminal tract beyond the polyQ.

O-200

Kinetics of protein aggregation

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The aggregation of proteins into fibrillar nanostructures is a general form of behaviour encountered for a range of different polypeptide chains. The formation of these structures is associated with pathological processes in the context of Alzheimer's and Parkinson's diseases but is also involved in biologically beneficial roles which include functional coatings and catalytical scaffolds. This talk focuses on recent work directed at understanding the kinetics of this process through the development and application of experimental biophysical methods and their combination with kinetic theories for linear growth phenomena.

P-201

Effect of 14-3-3(eta) on alpha-synuclein aggregation intermediates

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Alpha-synuclein (AS) is a neuronal protein of unknown function which is mainly involved in the pathophysiology of neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease and Dementia with Lewy Bodies (LBs). LBs contains not only AS, but also other proteins including 14-3-3 proteins. 14-3-3 proteins exist mainly as a dimer and its exact functions are remain unclear. However, recent work has shown that the association of 14-3-3(eta) with AS in LBs. Herein we show how 14-3-3(eta) can modulate AS *in vitro* aggregation behavior, by rerouting it toward the formation of stable non-fibrillar aggregates. We also show that the resulting populations of fibrillar and pre-fibrillar aggregates exhibit a modified toxicity in vivo with respect to the unperturbed aggregates. Interestingly, 14-3-3(eta) does not show any binding affinity for monomeric AS, nor for the mature fibrillar aggregates. We provide evidence that it acts on the oligomeric species which form during the amyloidogenesis process of aggregation. Since 14-3-3(eta) can influence the toxicity of amyloidogenesis without perturbing the functional AS monomers, we are convinced that once fully understood, its mode of action could represent a promising model to mimic with synthetic drugs and peptides.

P-202

What makes an amyloid toxic: morphology, structure or interaction with membranes?

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More than 40 human diseases are related to amyloids. In order to understand why some amyloids may become toxic to their host and some others are not, we first developed a genetical approach in the yeast *Saccharomyces cerevisiae*. We have chosen the amyloid/prion protein HET-s Prion

Forming Domain (218-289) from the fungi *Podospora anserina*, which is not toxic in yeast. Some toxic amyloids mutants were generated by random mutagenesis. *In vitro* the most toxic mutant called "M8" displays very peculiar nanofibers, which polymerized mainly in amyloid antiparallel β-sheets whereas the non-toxic WT exhibits a parallel polymerization. We further established the dynamic of assembling of the M8 toxic amyloid, in comparison to the WT non-toxic amyloid, and showed the presence of specific oligomeric intermediates also organized in antiparallel β-sheet structures. A more global structure/toxicity study on more than 40 mutants clearly identified an antiparallel β-sheet signature for all the toxic mutants. Therefore size, intermediates and antiparallel structures may account for amyloid toxicity in yeast but we still wonder what their cellular targets are. Recently, we established the first evidences that toxic mutants may specifically bind *in vitro* to lipids, particularly negatively charged.

P-203

Interconnected mechanisms in Abeta(1-40) peptide fibril formation

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We present an experimental study on the fibril formation of Aβ(1-40) peptide at pH 7.4. The kinetics of this process is characterized by the occurrence of multiple transient species that give rise to final aggregates whose morphology and molecular structure are strongly affected by the growth conditions. To observe in details the aggregation pathway as a function of solution conditions, we have used different experimental techniques as Light scattering, Thioflavin T fluorescence, Circular Dichroism and two-photon fluorescence microscopy. This approach gives information on the time evolution of conformational changes at molecular level, on the aggregates/fibrils growth and on their morphologies. The selected experimental conditions allowed us to highlight the existence of at least three different aggregation mechanisms acting in competition. A first assembly stage, which implies conformational conversion of native peptides, leads to the formation of small ordered oligomers representing an activated conformation to proceed towards fibril growth. This process constitutes the rate limiting step for two distinct fibril nucleation mechanisms that probably implicate spatially heterogeneous mechanisms.

P-204

Amyloid formations and interactions

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The formation of amyloid fibrils of amylin 10-29 was studied by means of molecular dynamics (MD) and energy partition on three peptide ß-sheet stack systems with the same amino acid composition: wild type amylin 10-29 (**Amyl 10-29**), reverse amylin 10-29 (**Rev-Amyl 10-29**) and scrambled amylin version **Scr-Amyl 10-29**. The results show that for Amylin10-29 peptides, amino acid composition determines the propensity of a peptide to form amyloid fibrils independent of their sequence. The sequence of amino acids defines the shape and the strength of amyloid protofibril, which conforms with the atom force microscope (AFM) data [1]. MD show that the 6x6RevAmyl-10-29 stack has looser self-assembly than the 6x6Amyl-10-29 stack, which conforms with the results of Fourier transform infrared spectroscopy (FTIR) measurements for the peptides studied [1]. The results of MD show that 6x6Amyl-10-29 could have a turn, which consists with FTIR data [1].

Acknowledgments
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O-205

Mechanistic insights into Aβ42 aggregation and effects of inhibitors

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Data on Aβ aggregation kinetics have been characterized by a large spread between experiments on identical samples that contain so many molecules that stochastic behaviour is difficult to explain unless caused by uncontrolled amounts of impurities or interfaces. We have therefore spent considerable effort to eliminate sources of inhomogeneity and reached a level of reproducibility between identical samples and between experiments on separate occasions that we can now collect data that can lead to mechanistic insights into the aggregation process per se, and into the mechanism of action of inhibitors. Data on Aβ42 aggregation will be shown that give insight into the influence of physical parameters like peptide concentration, shear and ionic strength, as well as the effect of inhibitory proteins, model membranes and the effects of sequence variations. Monte Carlo simulations of amyloid formation from model peptides corroborate the finding from experiments and underscore that the very high level of predictability and reproducibility comes from multiple parallel processes.

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P-206
Lipid-protein interaction and amyloid-like fiber formation: FLIM and FCS studies
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Negatively-charged membranes were reported to catalyze "amyloid-like" fiber formation by non-amyloidogenic proteins [1]. Our study aims to elucidate the factors that govern the formation of these amyloid-like fibers. Lysozyme was selected as a model of non-amyloidogenic protein and was fluorescently-labeled with Alexa Fluor 488 (A488-Lz). First, A488-Lz partition towards phosphatidylserine-containing liposomes was characterized quantitatively using fluorescence correlation spectroscopy (FCS), in order to calculate the protein coverage of liposomes. Secondly, the interaction between A488-Lz and negatively-charged lipid membranes was studied using both steady-state and time-resolved fluorescence techniques. This interaction was found to switch from a peripheral binding to the anionic headgroups, at high lipid/protein molar ratio (L/P), to a partial insertion of protein into the hydrophobic core of the membrane, at low L/P. Finally, the lipid-protein supramolecular complexes formed at low L/P were characterized by fluorescence lifetime imaging microscopy (FLIM). The mean lifetime of A488-Lz in these supramolecular structures is much lower compared to the values obtained for the free and bound A488-Lz at high L/P. The fiber characterization will be complemented by FCS studies.

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P-207
Zinc modulates copper coordination mode in prion protein octa-repeat subdomains
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We present the results of recent XAS measurements carried out on various portions of Prion-protein tetra-octarepeat peptides in complex with metals with the aim of elucidating the mechanism underlying the competition for peptide binding when two distinct metal ions (in our case Cu^{2+} and Zn^{2+}) are simultaneously present in solution. Thanks to the ability of the XAS technique in providing detailed local structural information of the metal site, we are able to demonstrate that Cu and Zn interact with the peptide, in the sense that increased Zn (Cu) concentration progressively changes the way Cu (Zn) is bound to the tetra-octarepeat, even if none of the two metals is ever able to completely displace the other.
We propose a structural model in which the $Cu^{2+}(Zn^{2+})$ coordination mode depends on the $[Cu^{2+}]([Zn^{2+}])$:[octarepeat] concentration ratio. The model is in agreement with the EPR results published by one of the authors (G.M.). Our picture suggest a mechanism of cross-regulation between Cu^{2+} and Zn^{2+} that is worth further investigating in other biological systems, like for instance in the case of the Aβ-peptides involved in the Alzheimer disease. A competition mechanism of the kind we propose may represent an additional general cell strategy for fine metal binding regulation.

O-208
Membrane permeabilization by purified soluble β-enriched oligomers of prion protein
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The conversion of normal PrP^{C} to its pathological isoform PrP^{Sc} is a key event in prion diseases and is proposed to occur at the cell surface or more probably in acidic late endosomes. A convergence of evidence strongly suggests that the early events leading to the structural conversion of the PrP seem to be in relation with more or less stable soluble oligomers, which could mediate neurotoxicity. As commonly shared by other amyloidogenic proteins, membrane-bound monomers undergo a series of lipid-dependent conformational changes, leading to the formation of oligomers of varying toxicity rich in β-sheet structures (annular pores, amyloid fibrils). Here, we have used a combination of biophysical techniques (Dynamic and Static Light Scattering, Fluorescence techniques, and Quartz Crystral Microbalance) to elucidate the interaction of native monomeric PrP and that of purified β-rich oligomeric PrP on model lipid membranes. Under well established conditions, three β-sheet-rich oligomers were generated from the partial unfolding of the monomer in solution, which were found to form in parallel. From single mutation and/or truncation of the full length PrP, the polymerization pathway is strongly affected, revealing the high conformational diversity of PrP. In our previous work, we identify the minimal region of the PrP protein leading to the same polymerization pattern of the full length PrP. Soluble 12-subunits and 36-subunits oligomers were obtained depending on the single mutation or truncation and purified. We compare their structural properties (FTIR, CD) when associated with anionic lipid bilayers and study their propensities to permeabilize the membrane. Fluorescence kinetics suggest different mechanisms of membrane perturbation for the monomer and the PrP oligomers. Deciphering this complex network of lipid interactions and conformational diversity of the PrP protein will help for understanding of how amyloidogenic proteins induce neurotoxicity.

P-209
Towards a dynamic vision of amyloid-membrane assemblies
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The traditional view of the lipid bilayer described as a "sea" of lipids where proteins may float freely, is going to be inadequate to describe the increasingly large number of complex phenomena which are known to take place in biological membranes. Membrane-assisted protein-protein interactions, formation of lipid clusters, protein-induced variation of the membrane shape, abnormal membrane permeabilities and conformational transitions of membrane-embedded proteins are only a few examples of the variegated ensemble of events whose tightly regulated cross-talk is essential for cell structure and function.
Experimental work on the above mentionated problems is very difficult and some time not accessible, especially when the studied systems have a fast dynamics.
Due to the large size of the systems usually involved in this multifaceted framework, a detailed molecular description of these phenomena is beyond the possibilities of conventional Molecular Dynamics techniques. It is thus necessary to approach new computational models on a mesoscopic scale that if, on one hand, overlook atomistic details, allow to capture phenomena that are thought to occur in spatial and time scales far beyond what is normally sufficient to describe small molecules. In this light we have endeavored in our lab an extensive campaign focused on coarse grained (CG) models of different lipid-protein assemblies. Simulations have been performed by the GROMACS simulation package and the standard MARTINI force field.
Several examples of lipid-protein assemblies involved in some amyloidoses are presented with the aim of evidencing the potential of this multiscale approach.

P-212
Phytoalexins in prevention of amyloid aggregation
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Amyloid aggregation, a generic behavior of proteins, is related to incurable human pathologies - amyloid-related diseases, associated with formation of amyloid deposits in the body. All types of amyloid aggregates possess rich β-sheet structural motif. The recent data confirm the toxic effect of aggregates on the cells, however, it was found that reduction of amyloid aggregates plays important role in prevention as well as therapy of amyloidosis. We have investigated effect of phytoalexin derivatives on amyloid fibrillization of two proteins, human insulin and chicken egg lysozyme, by ThT and ANS fluorescence assays. We have found that amyloid aggregation of both studied proteins was significantly inhibited by phytoalexin derivates cyclobrassinin and benzocamalexin. For most effective phytoalexins the estimated IC_{50} values were at low micromolar concentration. The observed inhibiting activity was confirmed by transmission electron microscopy. Our data suggest the potential therapeutic use of the most effective phytoalexins in the reduction of amyloid aggregation.
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O-213
Mechanistic insights into oligomeric alpha-synuclein/membrane interactions
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The amyloid pore hypothesis suggests that interactions of oligomeric alpha-synuclein (aS) with membranes play an important role in Parkinson's disease. Oligomers are thought to permeabilize membranes and interfere with Ca^{2+} pathways. Permeabilization by aS requires the presence of negatively charged phospholipids. Whether aS can bind and permeabilize membranes with physiologically relevant lipid compositions has not been extensively explored.
Here we report on the binding of aS to giant unilamellar vesicles (GUVs) with physiologically relevant lipid compositions. Comparing different protocols of oligomer preparation, leakage assays on both large unilamellar vesicles (calcein release) and GUVs (HPTS efflux assay) show that aS is not able to permeabilize these membranes. The presence of cholesterol has a stabilizing effect on these membrane systems. In agreement with these findings, we do not observe concentration dependent aS toxicity using *in vivo* MTS assays. However, in the calcein release assay, different aS preparations show differences in kinetics and aS concentrations that cause 100% leakage. These results motivate us to critically reassess the amyloid pore hypothesis, and suggest that membrane permeabilization may be attributable only to a very specific as species.

P-214
Alpha-synuclein oligomers impair membrane integrity—a mechanistic view
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One of the most prevalent neurodegenerative diseases is Parkinson's disease (PD), which is accompanied with the

loss of dopaminergic neurons. Although the mechanisms leading to the death of these cells are still unclear, the protein alpha-synuclein (aS) is one of the pivotal factors. Previous studies indicate that especially oligomeric forms of aS show a detrimental effect on membrane integrity. As an intact membrane is crucial to many cellular processes, the impairment of the membrane integrity is a likely pathway for neuronal death.

We use different phospholipid bilayer model systems to investigate the mechanisms underlying this process. Atomic force microscopy in combination with suspended asymmetric phospholipid bilayers, which closely mimic the plasma membrane, allows the identification of the binding sites, the measurement of penetration depths of the aS oligomers into the phospholipid bilayer, and the detection of membrane thinning or creation of membrane defects. Using an approach based on phospholipid vesicles we were able to observe for the first time that aS oligomers cause an enhanced lipid-flip flop. Suggesting that the loss of lipid asymmetry is a novel mechanism which may contribute to or trigger neuronal death in PD.

P-215

Amyloid protein-membrane interactions and structure-toxicity relationship study

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A better characterization of toxic amyloids would allow getting better insights into neurodegenerative diseases. What makes an amyloid toxic is, however, still unclear.

Our study aims to investigate the membrane interaction with toxic and non-toxic amyloids. We used the Prion Forming Domain PFD218-289 of the protein HET-s (WT) (*Podospora anserina)* and its toxic mutant M8 (generated by PCR random mutagenesis) as amyloid models. *In vitro*, they exhibit different fibril characterizations and secondary structures (Berthelot *et al.* FASEB J. 2009, 23, 2254).

At first, the interactions between WT and M8 with different charged phospholipid (DOPI, DOPG, DOPC, DOPS and DOPE) monolayers were highlighted. Results indicated that M8 (toxic) has a much higher and more specific effect on negatively charged phospholipids (DOPS, DOPI and DOPG) than in the case of WT (non-toxic). Therefore the insertion of protein into phospholipid monolayers, which occurred similarly for both WT and M8, is not a key factor for these effects (H.P. Ta *et al.* Langmuir, in press).

We are now using unilamellar vesicles as a membrane model to investigate the amyloid protein (toxic and non-toxic) – phospholipid interactions. Results confirmed the high specific and strong effects of M8 on negatively-charged membrane.

P-216

Investigations on alpha-synuclein pore forming properties

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Alpha-synuclein (aS) is a natively unfolded protein widely expressed in neurons at the presynaptic level, partially bound to synaptic vesicles. It is linked to Parkinson's disease (PD) by two lines of evidence: aS amyloid fibrils accumulate in patients' brains and 4 genetic mutants cause autosomal dominant forms of PD. The biological role of the protein and the mechanisms involved in the pathogenesis of PD are still unknown. Membrane binding of aS monomers causes the acquisition of an amphipatic alpha-helix, which lies on the surface without crossing the bilayer. Recent observations reported that a pore-like activity of aS can be recorded upon the application of a potential. This activity is characterized by defined conductance levels with a determined fingerprint in Planar Lipid Membrane (PLM) experiments. As several studies found aS oligomers are the most toxic species in cell cultures, we aim to use TIRF imaging and the PLM technique to give more information about the pore activity of aS. Particular effort is put into understanding whether monomeric and oligomeric aS share a similar mechanism for membrane permeabilization and to find possible implications for the ethiopatogenesis of PD. (Sponsored by Provincia Autonoma di Trento, Marie Curie Action COFUND, project SingleSyn)

P-217

Encapsulation and study of fibrillar networks in nanocompartments

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In this project, we study the chemical, physical and biological properties of fibrillar networks. The formation and the network mechanics are investigated by combining droplet-based microfluidics with optical microscopy and small angle x-ray scattering (SAXS).

The chosen system, fibrin network formation, plays an important role in blood coagulation processes. Crosslinking of fibrinogen induced by an enzymatic reaction with thrombin leads to 3D fibrin network formation.

The fibrillar networks are formed within picoliter droplets of aqueous solutions in an continuous oil phase. Droplets containing fibrinogen and thrombin can be produced of different sizes and stored for fibrin network formation. The formation of the fibrillar networks is imaged by fluorescence microscopy. To analyze the elastic properties of the networks, the droplets flow through a microchannel device of alternating width in order to squeeze and stretch the

networks. Additionally, SAXS experiments will give structural information about the molecular dimensions of the networks.

O-218

Biophysical consideration of gamma-secretase modulation as potential target for Alzheimer's disease

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The amyloid beta peptide (Aβ), implicated in Alzheimer's disease (AD), is released from the Amyloid Precursor Protein (APP) by secretase-induced cleavage. This process results in the release of a range of Aβ peptides varying in length. The brains of AD patients often contain longer Aβ peptides while the total concentration of Aβ is unaffected. Longer peptides are more hydrophobic having far-reaching consequences for their toxicity and aggregation. As Aβ is necessary for normal neuronal function, research activities into AD therapeutic development currently explore the possibilities of modulating γ-secretase activity to produce short Aβ peptides. Whether such an approach effectively ameliorates the toxic effect of Aβ has not been explored yet. To answer this question, we studied the impact of heterogeneity in Aβ pools in an *in vitro* biophysical and *in cellulo* context using MicroElectrode Array to assay the synaptic activity of primary neurons. We show that various lengths of the Aβ peptide and mixtures thereof aggregate with distinct kinetics and notoriously affect synaptotoxic and cytotoxic response. We also show that small amounts of less abundant peptides Aβ38 and Aβ43 induce aggregation and toxicity of Aβ40 while the behavior of Aβ42 is unaffected.

O-219

Microscopic analysis of protein oxidative damage: effect of carbonylation on structure, dynamics and aggregability of villin headpiece

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One of the most important irreversible oxidative modifications of proteins is carbonylation, a process of introducing the carbonyl group in reaction with reactive oxygen species. Importantly, carbonylation increases with the age of cells and is associated with the formation of intracellular protein aggregates and the pathogenesis of age-related disorders such as neurodegenerative diseases and cancer. However, it is still largely unclear how carbonylation affects protein structure, dynamics and aggregability on the atomic level. Here, we use classical molecular dynamics simulations to study structure and dynamics of the carbonylated headpiece domain of villin, a key actin-organizing protein. We perform an exhaustive set of molecular dynamics simulations of native villin headpiece together with every single combination containing carbonylated versions of its seven lysine, arginine and proline residues, the quantitatively most important carbonylable amino acids. Surprisingly, our results suggest that high levels of carbonylation, far above those associated with cell death *in vivo*, may be required to destabilize and unfold protein structure through the disruption of specific stabilizing elements, such as salt bridges or proline kinks, or tampering with the hydrophobic effect. On the other hand, by using thermodynamic integration and molecular hydrophobicity potential approaches, we quantitatively show that carbonylation of hydrophilic lysine and arginine residues is equivalent to introducing hydrophobic, charge-neutral mutations in their place, and, by comparison with experimental results, demonstrate that this by itself significantly increases intrinsic aggregation propensity of both structured, native proteins and their unfolded states. Finally, our results provide a foundation for a novel experimental strategy to study the effects of carbonylation on protein structure, dynamics and aggregability using site-directed mutagenesis.

P-220

Conformational and aggregation studies of *Schistosoma mansoni* septins

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Septins are an evolutionarily conserved family of GTP-binding proteins involved in important cellular processes, such as cytokinesis and exocytosis, and have been implicated in neurological diseases, such as Alzheimer's and Parkinson's diseases. The focus of this study was two septins of *Schistosoma mansoni,* (the causative agent of Schistosomiasis in South America) named Smsept5 and Smsept10, which were produced in a recombinant system. Our objective was to verify if these septins from a simpler organism display similar characteristics to human septins. Analysis of protein structure by Circular Dichroism showed that both recombinant SmSeptins produced were folded. The GTPase activity assay showed that Smsept5 was able to hydrolyze GTP, whereas Smsept10 was not. Aggregation studies for amyloid fibril detection by right angle light scattering and Thioflavin T fluorescence assay were performed. Both proteins showed a temperature dependent increase in light scattering and fluorescence emission of ThT probe. This indicated that *S. mansoni* septins tend to aggregate into amyloid-like fibers in high temperatures, with thresholds of 30 °C for Smsept5 and 37 °C for Smsept10. These results are in accordance to that previously reported for human septins.

Nucleic acid and chromatin structure & function

P-222
Efficacy of DNA repair mechanisms in lymphocytes of melanoma patients as prognostic factor of chemotherapy
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In our work we investigated the response to standard chemotherapy of blood lymphocytes of patients suffering with melanoma. DNA single and double strand breaks were determined using comet assay; intracellular levels of marker proteins were detected using immunocytochemistry. Ultimately this set of parameters allows to characterize two mechanisms of DNA repair (base excision repair, BER and mismatch repair, MMR) which together with apoptosis proneness underlie response of tumor cells to chemotherapy. Cell death caused by O^6mG adducts is promoted by MMR system by inducing unrepaired double strand breaks in DNA. There was a linear correlation between the level of dsDNA breaks in lymphocytes after 1-st cycle of chemotherapy and MMR efficiency in them.
The level of double strand breaks in DNA after 1-st cycle of chemotherapy is predictive of clinical outcome. Otherwise damage at the level of ssDNA (AP-sites and single strand breaks) and BER mechanism associated with it couldn't be a good prognostic factor of chemotherapy. High level of double strand breaks in DNA in blood lymphocytes of melanoma patients 48 hours after 1-st cycle of chemotherapy appears to be a marker of a good prognosis.

P-223
Self-assembly and stability of g-quadruplex: counter-ions, pressure and temperature effects
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The important role of G-Quadruplex in biological systems is based on two main features: composition and stability of telomeres, and activity of telomerase. The G-quadruplex structures are formed by supramolecular organization of basic units called G-quartets that are planar rings constituted by four guanosines linked by Hoogsten hydrogen bonds. GQuadruplex requires the presence of monovalent cations playing a key role in stabilizing these structures, since they give rise to coordination bonds needed for the stacking of more tetrads. We performed X-ray diffraction experiments at different pressures (ranging from 1 to 2000 bar), and small angle X-ray scattering (SAXS) changing the temperature (between 20-60°C). Diffraction results concerning samples with an intermediate water content (50-40% H2O) evidence the presence of two phases (Cholesteric and Hexagonal phase), while more concentrated samples (30-20% H2O) present just the hexagonal phase. High-pressure experiments provided the evidence that compression increases the system disorder without phase transitions. Data obtained from SAXS measurements show that increasing concentration of KCl we obtain more stable and larger particles. Also the temperature stability of the aggregates increases at increasing counter-ions content.

O-225
Nucleosome accessibility investigated using single molecule fluorescence
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Nucleosomes are the smallest compaction unit of chromatin in the cell nucleus. They consist of 150 bp DNA wrapped around a protein core of two (H2A-H2B) histone dimers and a (H3-H4)2 tetramer. DNA in nucleosomes is sterically occluded and nucleosomes must open to allow full DNA access. We studied this process using FCS and single molecule FRET. Here we show evidence for a previously uncharacterized intermediate structural state that preceeds histone dissociation, in which the distance between H2B and the nucleosomal dyad is increased. Our data suggest that the first step in nucleosome disassembly is the opening of the (H3-H4)2 tetramer/(H2A-H2B) dimer interface, followed by H2A-H2B dimer release from the DNA and, lastly, (H3-H4)2 tetramer removal. We estimate that the open intermediate state is populated at 0.2 - 3 % under physiological conditions, and could have significant in vivo implications for factor-mediated histone exchange, as well as for regulating DNA accessibility to the transcription and replication machinery. Additionally, histone variants and histone modifications could substantially change the stability of the nucleosome. Our most recent spFRET data indicate that such effects may occur both at the level of DNA-histone and histone-histone interactions.

O-226
Dissecting the role of coregulator exchange and chromatin binding in retinoic acid receptor (RAR) mobility by live cell FCS
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Retinoic acid receptor (RAR) is a member of the nuclear receptor superfamily. This ligand-inducible transcription factor binds to DNA as a heterodimer with the retinoid X receptor (RXR) in the nucleus. The nucleus is a dynamic compartment and live-cell imaging techniques make it possible to investigate transcription factor action in real-time. We studied the diffusion of EGFP-RAR by fluorescence correlation spectroscopy (FCS) in order to uncover the molecular interactions determining

receptor mobility. In the absence of ligand we identified two distinct species with different mobilities. The fast component has a diffusion coefficient of D_1 = 1.8-6 $\mu m^2/s$ corresponding to small oligomeric forms, whereas the slow component with D_2 = 0.06-0.12 $\mu m^2/s$ corresponds to interactions of RAR with the chromatin or other large structures. The RAR ligand binding domain fragment also has a slow component probably as a result of indirect DNA-binding via RXR, with lower affinity than the intact RAR:RXR complex. Importantly, RAR-agonist treatment shifts the equilibrium towards the slow population of the wild type receptor, but without significantly changing the mobility of either the fast or the slow population. By using a series of mutant forms of the receptor with altered DNA- or coregulator-binding capacity we found that the slow component is probably related to chromatin binding, and that coregulator exchange, specifically the binding of the coactivator complex, is the main determinant contributing to the redistribution of RAR during ligand activation.

P-227

Compactization of DNA induced by sperm-specific proteins of H1 family

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Formation of inactive nuclear with high level of DNA compaction in sperm cells is accompanied by a substitution of linker histones H1 by a number of other proteins. Among them sperm-specific histones (SSH), which are characterized by elongated arginine-rich polypeptide chain compared to the somatic H1. The secondary and tertiary structure of the SSH and their interactions with DNA were studied using spectroscopic and thermodynamic approaches. The histones were isolated from sperm of marine invertebrates and rat thymus. All studied SSH demonstrate no considerable compaction of DNA in solutions of low ionic strength. However, at physiological conditions, SSH H1 from *S.intermedius* and *A.japonica* compact DNA more intensively than other SSH. The somatic H1 from rat thymus revealed a minimal ability to compact the DNA. We suggest that the SSH H1 are able to interact with DNA not only in the major groove but also in the minor groove of the double helix inducing considerable structural changes in DNA and facilitating the formation of the supercompact sperm chromatin.

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P-228

Investigation of radiation-induced damages in DNA bases and secondary structure

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Ionizing radiation causes modification and destruction of nitrogenous bases in DNA molecule. There are also local breakages of hydrogen bonds both in the lesion sites mentioned above and in other sites of the macromolecule. To reveal the amount of some of these damages we applied CD and UV absorption spectroscopy. Radiation-induced changes in DNA structure influence its UV absorption spectrum in different ways: partial denaturation causes hyperchromic effect, while destruction of the bases results in hypochromic shift. At the same time both of them result in the same changes in DNA CD spectra: the decrease in intensity. We attempted to segregate the described damages in DNA structure and studied the influence of DNA ionic surroundings on the radiation effect. It is shown that the radiation efficiency of base destruction and partial denaturation increases with decreasing concentration of NaCl in irradiated solution. The substitution of a portion of Na^+ ions on Mg^{2+} with the total ionic strength remaining constant (0.005 M) does not influence the radiation efficiency.

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P-229

Udu and its homologs, Udu2 and Gon4l, may have similar functions in DNA replication

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Udu (Ugly duckling) has been first identified from a zebrafish mutant and shown to play an essential role during erythroid development; however, its roles in other cellular processes remain largely unexplored. FACS analysis showed that the loss of *udu* function resulted in defective cell cycle progression and comet assay indicated the presence of increased DNA damage in *udu* mutants. We further showed that the extensive p53-dependent apoptosis found in *udu* mutants is a consequence of activation in the Atm-Chk2 pathway. Udu appears not to be required for DNA repair, because both wild-type and *udu* embryos similarly respond to and recover from UV treatment. Yeast two-hybrid and coimmunoprecipitation data demonstrated that PAH-L repeats and SANT-L domain of Udu interacts with MCM3 and MCM4. Furthermore, Udu was colocalized with BrdU and heterochromatin during DNA replication, suggesting a role in maintaining genome integrity.

Recently, we started to work on the second zebrafish homolog, Udu2, and its mammalian counterpart, Gon4l. Preliminary data showed that *udu2* and *Gon4l* mRNA injection can rescue zebrafish *udu* mutant phenotypes. Furthermore, PAH-L and SANT-L domains of Udu2 and Gon4l can bind to MCM3 and MCM4 and they are localized in the nucleus. These data suggest that Udu2 and Gon4l are functionally equivalent to zebrafish Udu. Their molecular mechanism leading to *udu* phenotypes is currently under investigation.

O-230

Chromatin condensation: general polyelectrolyte association and histone-tail specific folding

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The major component of chromatin, DNA, is a densely charged polyanion. Electrostatic interactions between DNA and DNA-packaging proteins contribute decisively to formation of its elementary unit, the nucleosome, and are also important in chromatin folding into higher-order structures. We investigate condensation of DNA and chromatin and find that electrostatics and polyelectrolyte character of DNA play dominant role in both DNA and chromatin condensation. By comprehensive experimental studies and using novel oligocationic ligands, we suggest simple universal equation describing DNA condensation as a function of oligocation, DNA and monovalent salt concentrations and including the ligand-DNA binding constant. We found that similar dependence was also observed in condensation of the nucleosome arrays. Next, we studied how general electrostatic and specific structural alterations caused by lysine acetylations in the histone tails influence formation of 30-nm chromatin fibre and intermolecular nucleosome array association. For the first time, a structural model is suggested which explains critical dependence of chromatin fibre folding on acetylation of the single lysine at position 16 of the histone H4. Exceptional importance of the H4 Lys 16 acetylation in general and gene specific transcriptional activation has been known for many years but no structural basis for this effect has yet been proposed.

O-232

Lambda genetic switch sensitivity depends on complex looping kinetics driven by nonspecific binding

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The kinetics of DNA loop formation and breakdown by the l repressor protein (CI) have been characterized at various concentrations of protein using the tether particle motion (TPM) technique and a novel method of analysis with increased time resolution. Comparison of the kinetics of looping in wild-type DNA or DNA with mutated operators, showed that repressor bound at these sites may nucleate adjacent CI binding to further stabilize loops and broaden the distribution of rate constants for loop formation and breakdown. The average activation energies were calculated from the rate constant distributions and are consistent with a model in which nonspecific binding of CI along the segment between the operators shortens their effective separation, thereby lowering the energy barrier for loop formation. The interplay of this effect with the enhanced loop stability at increased CI concentration is crucial for loop efficiency since it allows to keep constant the frequency of transition from the looped to the unlooped DNA conformation at varying CI concentration. Thus at increased CI concentrations, although the loop became more stable thermodynamically, it periodically opens and thus remains sensitive to changing environmental conditions which may require switching to lytic conditions.

P-233

Surface-enhanced Raman scattering of genomic DNA from apple leaf tissues

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Detection of specific DNA sequences is central to modern molecular diagnostic. Ultrasensitive Raman measurements of nucleic acids are possible through exploiting the effect of surface-enhanced Raman scattering (SERS). In this work, the SERS spectra of genomic DNAs from leaves of different apple trees grown in the field, have been analyzed [1].
A detailed comparative analysis of SERS signatures of genomic DNAs is given. SERS wavenumbers (cm^{-1}) are reported here for all types of vibrations of plant genomic DNAs, including bands assigned to localized vibrations of the purine and pyrimidine residues, localized vibrations of the deoxyribose-phosphate moiety, etc. Proposed band assignments are given. A strong dependence of the SERS spectra on DNA concentration and on time have been observed.
In biochemical fields, nucleic acids might be used to explore the interaction between DNA and small molecules, which is important in connection with probing the accurate local structure of DNA and with understanding the natural DNA-mediated biological mechanisms [2].

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P-234

The pH-dependent structure of DNA studied by Fourier Transform Infrared Spectroscopy

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Fourier transform infrared (FT-IR) spectra of deoxyribonucleic acid have been obtained and analyzed in the wide range of pH (2.9-10.2) environmental conditions.

The region of the infrared spectrum studied covered the wave number range from 4000 cm^{-1} to 700 cm^{-1}. IR spectra show that in pH 8.0-9.0 interval carbonyl (C=O) band at 1689 $^{-1}$cm (assigned to guanine) is reduced in intensity and slightly shifted to lower frequencies. At the pH 10.0 significantly decreases band intensity at 1712 cm^{-1} due to unbounded C2=O of Thymine and shifts to lower frequencies, indicating at the transition of this group in bounded form, supposedly by means of excess polarized hydroxyl ions. Together this, in basic region a new intense absorption band has been observed in 1500-1300cm^{-1} frequency interval, corresponding to O-H group in-plane bending vibration (1410-1310cm^{-1}).
As for acidic conditions, it was observed that under the extreme pH ($\sim$2.8) value carbonyl absorption region shifts to higher frequencies and absorption intensity significantly increased, indicated at releasing of C=O groups from H-bonding between base pairs. Moreover, bands intensity at 1418cm^{-1} and 1022cm^{-1} corresponding to out-of-plan deformation of NH_2 groups increased due to rupture of connections between the DNA strands.

P-235
Structural order in complexes of DNA with chromosomal proteins HMGB1 and H1
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During the last decade it was found that in many cases specific structural organization of multi-molecular protein and DNA-protein complexes determines their functioning in living cells. Although these functioning structures are usually unique, it is often possible to identify their common structural elements. One of the interesting examples of such universal elements are HMGB domains: structurally conservative functional domains of non-histone proteins HMGB1/2 also identified in many nuclear proteins. Using AFM, thermodynamic approaches, circular dichroism and molecular absorption spectroscopy in far-UV and mid-IR regions we have studied structural organization of the complexes between DNA and different proteins, including HMGB1, HMGB-domain recombinant proteins and linker histone H1. We have demonstrated, that interaction with DNA leads to increasing both α-helicity of the proteins and thermal stability of DNA. Also, this interaction may result in formation of highly ordered supramolecular complexes facilitated by HMGB-domains. The C-terminal sequence of HMGB1/2 regulates affinity of the proteins to DNA and can be “inactivated” by interaction with histone H1. Based on the data obtained a model of the interaction of multy-domain HMGB-proteins with DNA is suggested.

O-236
DNA replication machinery clamps down on chromatin mobility
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Chromatin in living cells displays considerable mobility on a local scale. This movement is consistent with a constrained diffusion model, in that individual loci execute multiple, random jumps. To investigate the connection between local chromatin diffusion (LCD) and the changes in nuclear organization, we established a stable HeLa cell line expressing GFP-PCNA. This protein, a core component of the replication machinery, serves as a cell-cycle marker and allows us to visualize sites of ongoing DNA synthesis within the nucleus. To monitor LCD, we labeled discrete genomic loci through incorporation of Cy3-dUTP. This experimental system, in conjunction with particle tracking analysis, has enabled us to quantitatively measure chromatin mobility throughout the cell cycle.
Our results demonstrate that LCD is significantly decreased in S-phase. To explore the connection between DNA replication and reduced chromatin movement, we undertook a more detailed examination of LCD in S-phase nuclei, correlating chromatin mobility with sites of replication. Our results demonstrate that labeled chromatin in close proximity to GFP-PCNA foci exhibit significantly decreased mobility. We therefore conclude that presence of active replication forks constrains the movement of adjacent chromatin.

O-238
Single-molecule studies of DNA replication
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Advances in optical imaging and molecular manipulation techniques have made it possible to observe individual enzymes and record molecular movies that provide new insight into their dynamics and reaction mechanisms. In a biological context, most of these enzymes function in concert with other enzymes in multi-protein complexes, so an important future direction will be the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies.
We are applying a single-molecule approach to study DNA replication. I will present recent results of single-molecule studies of replication in bacterial and eukaryotic systems. By combining the stretching of individual DNA molecules with the fluorescence observation of individual proteins, we visualize the dynamic interaction of replication factors with the fork. In the bacteriophage T7 replication system, we show that DNA polymerases dynamically associate with and dissociate from the fork during replication. Further, I will present

new data from single-molecule replication studies in *X. laevis* oocyte extracts. We have developed a novel imaging scheme that permits single-molecule fluorescence experiments at concentrations of labeled protein that were hitherto inaccessible. Using this method, we visualize, in real time, origin firing and fork movement.

O-239

***In silico* single molecule manipulation with rigid body dynamics: an efficient tool for modeling the mechanical properties of DNA-protein complexes**

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Keywords: Single molecule manipulation, magnetic tweezers, rigid body dynamics, nucleosome, chromatin fiber.

Whereas Magnetic Tweezers (MT) and Optical Tweezers (OT) provide a unique tool for probing the mechanical properties of DNA-protein complexes, in particular those of nucleosomes, they remain of little help in characterizing chromatin fiber structure. As a matter of fact, force-extension diagrams require an underlying model for their interpretation.
We therefore have developed a new and powerful method for manipulating chromatin fibers under MT or OT *in silico*. Our algorithm is based on an open source library called ODE (Open Dynamics Engine, http://www.ode.org/) with which we have implemented a Langevin dynamics.
Force-extension diagrams of reference models of naked DNA (freely jointed chain, wormlike chain) as well as extension-rotation diagrams of naked DNA have been successfully recovered. Of note, plectonemic structures are most efficiently simulated thanks to ODE's collision detection code.
New insights into nucleosome and chromatin fiber structure and dynamics will be presented.

P-240

The study of the pKM.101 plasmid effect on the repair of DNA

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In our experiments was studied the effect of pKM.101 plasmid on repair of single strand breaks in DNA induced by 60Co-gamma irradiation in E.coli K 12 AB 1157 (wild type) and its different rec mutant cells. The pKM.101 resistant-factor in case of the control decreases the sensitivity of radiation, which as we suppose, is reached by the help of DNA conformation change. It can be supposed from the well known effect of radiation biology that by the effect of pKM.101, the ratio of DNA radiation sensitive volumes by appearing its new conformation decreases. The pKM.101 R-factor in rec mutants in case of gamma irradiation shows effects in two ways. One is the "chemical" connection between the R-factor and DNA, though the other relate to positive and negative "induced" radiation resistance from the local type effect of the connection of an R-factor and a rec mutant, and the two radiation resistant effects are added algebraically. As a result from the view of biology we have to categorize the radiation resistance and the connected repair processes as two different classes according to the change either in the chemical or in the induced radiation resistant effect.

Computational biophysics and simulation

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Dependence on membrane composition and amino acid sequence of antimicrobial peptides activity. A molecular dynamics simulation study

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Important antimicrobial properties are recognized in several peptides, for which the ability to interact and disrupt bacteria membranes have been reported. However, different mechanisms of action have been identified, suggesting that a complex interplay of different factors are in the origin of such behavior.
Recent studies have indicated that two trimethylated peptides (K6, K7), derived from the parental hybrid peptide CA(1-7)M(2-9), strongly interact with a bacterial membrane model (mixture of zwitterionic and negatively charged lipids), but not with a membrane model of mammalian erythrocytes (zwitterionic lipids) [1]. A reduction of the cytotoxicity effect and an improvement of the therapeutic index have also been reported for the derivatives when compared with the parental CA(1-7)M(2-9) [2].
In this work, with the aim of providing insight on the interaction phenomena of the indicated peptides with zwitterionic and negatively charged membrane models, a systematic molecular dynamics study was carried out. Full hydrated bilayers of DMPC:DMPG (3:1) and POPE:POPG(3:1) were studied in the presence of each peptide, and results analyzed in terms of peptide structure and membrane composition.
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Interlipid links in galactolipid and phospholipid bilayers. A comparative molecular modeling study

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Lipid-water and lipid-lipid interactions at the membrane/water interface play important role in maintaining the bilayer structure, however, this region is not easily available for experimental studies. We performed molecular dynamics simulations of two bilayers composed of two different types of lipids: (1) dioleoylphosphatidylcholine (DOPC); (2) galactolipid monogalactosyldiacylglycerol (MGDG). To investigate the properties of the membrane/water interface region, we performed analysis of lipid-lipid interactions: direct, via charge pairs (DOPC) and hydrogen bonds (MGDG) as well as indirect, via water bridges. We also examined water-lipid interactions. Existence of well-defined entities (lipids) linked by different types of interactions (hydrogen bonds, charge pairs, water bridges) makes the analysis of the membrane/water interface region a suitable for a graph theoretical description. We applied a network analysis approach for comparative analysis of simulated systems. We note a marked difference between the organization as well as the dynamics of the interfacial region of the two bilayers.

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Ligand chirality effects the dynamics of human 3-phosphoglycerate kinase

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L-nucleoside analogues form an important class of antiviral and anticancer drug candidates. To be pharmacologically active, they need to be phosphorylated in multiple steps by cellular kinases. Human phosphoglycerate kinase (hPGK) was shown to exhibit low specificity for nucleotide diphosphate analogues and its catalytic efficiency in phosphorylation was also affected. To elucidate the effect of ligand chirality on dynamics and catalytic efficiency, molecular dynamics simulations were performed on four different nucleotides (D-/L-ADP and D-/L-CDP) in complex with hPGK and 1,3-bisphospho-D-glycerate (bPG). The simulation results confirm high affinity for the natural substrate (D-ADP), while L-ADP shows only moderate affinity for hPGK. The observed short residence time of both CDP enantiomers at the active site suggests very weak binding affinity which may result in poor catalytic efficiency shown for hPGK with D-/L-CDP. Analysis of the simulations unravels important dynamic conditions for efficient phosphorylation replacing the single requirement of a tight binding.

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The electron correlation effects on the energy band structures of double stranded DNA

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Using the van der Waals density functional based on the semilocal exchange functional PW86 together with a long-range component of the correlation energy [1] implemented in the SIESTA program code, we have calculated the band structure of the double stranded DNA. The unit cell was built by taking together 10 GC (or AT) homogenous base pairs and we have considered the translational symmetry as the periodic boundary condition. The results obtained are compared with the oligomer calculations taking up to seven base pairs. The band structure obtained with this van der Waals density functional is also compared with results obtained with other exchange-correlation functionals as well as with band structure obtained by the Hartree-Fock crystal-orbital method taking into account the helical symmetry of the double stranded DNA. The role of different parts of DNA (base pairs, sugar-phosphate backbone, Na ions) is also presented.

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O-247

Using low-resolution structural data to model structure, function and motion in transmembrane proteins

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Transmembrane (TM) proteins comprise some 15% to 30% of the proteome but owing to technical difficulties, relatively few of these structures have been determined experimentally. Computational modeling techniques can be used to provide the essential structural data needed to shed light on structure-function relationships in TM proteins. Low-resolution electron-density maps, obtained from cryo-electron microscopy (cryo-EM) or preliminary X-ray diffraction studies, can be used to restrict the search in conformational space. At the right resolution, the locations of TM helices can be roughly determined even when the amino acids are not visible. When these data are combined with physicochemical characteristics of amino acids (such as their hydrophobicity) and with evolutionary conservation analysis of the protein family, the location of the amino acids can be modeled. The model-structure may provide molecular interpretations of the effects of mutations. Moreover, it can be used to suggest molecular mechanisms and to design new mutations to examine them. The overall approach will be demonstrated using two human proteins: copper transporter 1 (CTR1), which is the main copper transporter in the human cell, and the 18 kDa translocator protein (TSPO) of the outer mitochondria. Model-structures of these proteins and their functional implications in health and disease will be discussed.

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Mechanism of conductance and selectivity in a model calcium channel from Monte Carlo simulations

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Calcium channels play a crucial role in many physiological functions and their selectivity mechanism is still an unresolved question and a subject of debate. A physical model of selective "ion binding" in the L-type calcium channel is constructed, and consequences of the model are compared with experimental data. This reduced model treats only ions and the carboxylate oxygens of the EEEE locus explicitly and restricts interactions to hard-core repulsion and ion-ion and ion-dielectric electrostatic forces. According to the charge/space competition mechanism, the charge of structural ions attracts cations into the filter, while excluded volume effects are trying to keep them out. This is a competition between energy and entropy, where the balance of these terms minimizes free energy and determines selectivity. Experimental conditions involving binary mixtures of alkali and/or alkaline earth metal ions are computed. The model pore rejects alkali metal ions in the presence of biological concentrations of Ca2+ and predicts the blockade of alkali metal ion currents by micromolar Ca2+. Conductance patterns observed in varied mixtures containing Na+ and Li+, or Ba2+ and Ca2+, are predicted. Ca2+ is substantially more potent in blocking Na+ current than Ba2+. In apparent contrast to experiments sing buffered Ca2+ solutions, the predicted potency of Ca2+ in blocking alkali metal ion currents depends on the species and concentration of the alkali metal ion, as is expected if these ions compete with Ca2+ for the pore. These experiments depend on the problematic estimation of Ca2+ activity in solutions buffered for Ca2+ and pH in a varying background of ulk salt. Equilibrium binding affinity (expressed as the occupancy of the selectivity filter by various ions) is computed by equilibrium grand canonical Monte Carlo (GCMC) simulations. The conductivity of the channel is estimated from the equilibrium concentration profiles using the integrated Nernst-Planck equation. Our simulations show that the selectivity of L-type calcium channels can arise from an interplay of electrostatic and hard-core repulsion forces among ions and a few crucial channel atoms. The reduced system selects for the cation that delivers the largest charge in the smallest ion volume. We have also performed dynamic Monte Carlo (DMC) simulations for a model Ca channel to simulate current directly and present our results for the dynamical selectivity (expressed as the flux carried by various ions). We show that the binding affinity of Ca2+ versus Na+ is always larger than the dynamical selectivity because Ca2+ ions are tightly bound to the binding site of the selectivity filter of the channel and, at the same time, their mobility and drift velocity is smaller in this region.

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Vibrational Spectroscopy of Biomolecules by mixed Quantum / Classical Molecular Dynamics

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Carotenoids are used in light-harvesting complexes with the twofold aim to extend the spectral range of the antenna and to avoid radiation damages. The effect of the polarity and conformation of the environment is supposed to be responsible for the tuning of the electronic, optical and vibrational properties of peridinin carotenoid both in solution and in protein matrix. We investigate the effect of vibrational properties of peridinin in different solvents by means of vibrational spectroscopies and QM/MM molecular dynamics simulations[1]. The shift of vibrational fingerprints in the 1500-2000 cm^{-1} frequency region, due to the solvent polarity and proticity, is studied in three cases: cyclohexane (apolar/aprotic), deuterated acetonitrile (polar/aprotic) and methanol (polar/protic).

The frequencies and vibrational modes of the carbonyl, the allene, and the polyene chain were identified using effective normal mode analysis[2] and compared with the present and previous experimental data[3]. On the basis of our calculations and experiments in different solvents, we propose a classification of the four peridinins of the high-salt PCP form.

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P-250
Adsorbtion of cysteine on gold (111) surfaces: a DFT study
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The controlled self-assembly of functional molecular species on well defined surfaces is a promising approach toward the design of nanoscale architectures. By using this methodology, regular low-dimensional systems such as supramolecular clusters, chains, or nanoporous arrays can be fabricated. Small biological molecules such as amino acids represent an important class of building blocks that are of interest for molecular architectonic on surfaces because they inherently qualify for molecular recognition and self-assembly [1]. The interaction between amino acids and solid surfaces is decisive for the development of bioanalytical devices or biocompatible materials as well as for a fundamental understanding of protein-surface bonding.
We investigate the adsorbtion mechanism of the cysteine on Au(111) surfaces by means of the DFT [2]. Our main concern is to describe the molecule-metal bonding mechanism. Therefore we present a complex study, including the full determination of the density of states for the free and adsorbed molecule, the determination of molecule-surface bonding energy. The method of crystal orbital overlap populations is used in order to determine the contribution of specific atomic orbitals to the molecule-metal bond.

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P-251
Human myoglobins: paradigm of structure-function relation
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It is now widely accepted that myoglobin (Mb) is not simply an O_2 storage/delivery system but, depending on oxygen concentration, it exerts other fundamental physiological roles. Recent studies revealed a widespread expression and, in particular, an over-expression in response to hypoxia, in various non-muscle tissues, including tumor cells. In Human five different Mb isoforms are present. The two most expressed ($>$ 90%) differ only at the 54^{th} position, K54 (Mb-I) and E54 (Mb-II) respectively. Since high-altitude natives from Tibet are characterized by a higher Mb concentration and locomotion efficiency, together with the observation that the Mb overexpression is totally attributable to Mb-II, the idea that the latter might be one of the responses to high-altitude evolutionary adaptation, i.e. hypoxic environment, started to emerge. However, this is not yet supported by any structure/function investigation.
We performed hundred nanoseconds MD simulations on human Mbs to investigate the structure and dynamics of both protein and surface water. Important differences have been found at the histidine gate region, suggesting that the two more expressed human myoglobins might have a distinct and complementary role. While ^{1}H and ^{129}Xe nmR experiments do not show structural/dynamical differences, or not on longer time-scale than those offered by MD simulations, EPR experiments pointed out a different affinity of the two isoforms for NO, thus confirming a potential diverse role as predicted by simulations.
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P-252
Structural, dynamic and thermodynamic effects of KIT mutations: a computational multi-approach study
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Protein kinases play key roles in cell signaling and constitute crucial therapeutic targets. In normal cell, upon substrate binding, tyrosine kinase receptor KIT undergoes extensive structural rearrangement leading to receptor dimerization and activation. This process is initiated by the departure of the juxta membrane region (JMR) from the active site, allowing the activation loop (A-loop) deployment. The deregulation of KIT activity is associated with various forms of cancer provoked by abnormalities in signal transduction pathways. Mutations V560G (JMR) and D816H/V (A-loop) have been reported as oncogenic and/or drug-resistant. To contribute further in the understanding of KIT activation/deactivation mechanisms, we applied a multi-approach protocol combining molecular dynamics (MD), normal modes analysis (NMA) and pocket detection. Disturbing structural effects, both local (A-loop) and long-range (JMR), were evidenced for KIT D816H/V in the inactive state. nmA showed that JMR is able to depart its position more easily in the mutants than in the wild type. Pockets analysis revealed that this detachment is sufficient to open an access to the ATP binding site. Our results provided a plausible conception of mutant dimerization and a way to explore putative allosteric binding sites.

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Transmembrane association of leukocyte integrin heterodimer might be mediated by a polar interaction
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The lateral association of transmembrane (TM) helices is important to the folding of membrane proteins as well as a means for signaling across the cell membrane. For integrin, a hetero-dimeric protein important for cell adhesion and migration, the association of its α- and β-subunits' TM helices plays a key role in mediating bi-directional mechanical signaling across the membrane. We found evidence from experiment and simulation for a polar interaction (hydrogen-bond) across leukocyte integrin $\alpha L\beta 2$ TM that is absent in the better-studied platelet integrin $\alpha IIb\beta 3$ [1]. Our coarse-grained

molecular dynamics simulations of TM helix-helix self-assembly showed more native-like packing achieved by $\alpha L\beta 2$ within the simulation timescale as compared to its 'loss-of-function' $\beta 2$T686G mutant or $\alpha IIb\beta 3$ [2]. Association free energy profiles also showed a deeper minimum at a smaller helix-helix separation for $\alpha L\beta 2$, suggestive of tighter packing. The likely conservation of this polar interaction across the $\beta 2$ integrin family further reinforces its importance to the proper functioning of leukocyte integrins.

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Meropenem vs. Imipenem interacting with MexB: structural and dynamical determinants of the efflux action on two substrates

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Active extrusion of drugs through efflux pumps constitutes one of the main mechanisms of multidrug resistance in cells. In recent years, large efforts have been devoted to the biochemical and structural characterization of RND efflux pumps in Gram-negative bacteria, in particular the AcrB/A-TolC system of E.Coli. Specific attention has been addressed to the active part of the efflux system, constituted by the AcrB unit. Despite the presence of several data, crucial questions concerning its functioning are still open. The understanding of the structure-dynamics-function relationship of MexB, the analogous transporter in P. Aeruginosa, encounters even more difficulties, because of the lack of structural data of the transporter in complex with substrates. To shade some light on the activity of MexB, we performed computational studies on MexB interacting with two compounds, meropenem and imipenem, the first known to be a good substrate, and the second a modest one. Several techniques were used in the present work, ranging from flexible docking [1] to standard and targeted molecular dynamics (MD) simulations. Starting from the published crystal structure [2] we identified the most probable poses of the two compounds in both the original experimental and in the MD-equilibrated structures. We used information from AcrB binding pocket in order to find relevant binding sites of the two compounds in the analogous binding pocket of MexB.
Meropenem frequently lies with appropriate orientation in a pocket similar to the one identified for doxorubicin in AcrB [3], while the occurrence of imipenem poses in the same pocket is very scarce. Additionally, when present in the pocket, imipenem is located in a position that renders very unlikely its extrusion toward the OprM docking domain during the simulation of the functional peristalsis. The analysis of the trajectories has provided a complete inventory of the transporter and antibiotic hot spots, which is key information in terms of screening and design of antibiotics and inhibitors.

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P-255

Simulations of clathrin self-assembly into polyhedral cages

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Clathrins are three-legged proteins with the intriguing ability to self-assemble into a wide variety of polyhedral cages. The nucleation and growth of a clathrin lattice against the cytosolic face of a cell membrane enables the endocytosis of membrane proteins and various external molecules, by wrapping the membrane around the cargo to produce a coated transport vesicle within the cell. Clathrins can also self-assemble, in slightly acidic solutions devoid of auxiliary proteins, into empty cages. Our simulations of this process, using a highly coarse-grained model, indicate that the key to self-assembly is neither calthrin's characteristic puckered triskelion shape, nor the alignment of four legs along all cage edges, but an asymmetric distribution of interaction sites around the leg's circumference. Based on the critical assembly concentration, the binding strength in these cages is estimated at 25 to 40 k_BT per clathrin. The simulations also answer the long-standing conundrum of how flat patches of purely hexagonal clathrin lattices, which in cryo electron microscopy are frequently seen to decorate cell membranes, can produce highly curved cages containing twelve pentagonal faces interdispersed between hexagonal faces. We present experimental evidence supporting this pathway.

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Allostery in nuclear export: a hybrid methods approach

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In eukaryotic cells, the exchange of macromolecules between the cytoplasm and the nucleus is mediated by specialized transport factors. By binding to these transporters, cargo molecules, which are otherwise excluded from entering the nucleus, can traverse the nuclear pore complex efficiently. Most of the proteins mediating nuclear import and export exhibit a characteristic _-solenoid fold, which provides them with an unusual intrinsic flexibility. CRM1 is an essential nuclear export receptor, which recognizes a very broad range

of export cargoes. CRM1-dependent nuclear export is RanGTPase-driven, and recognition of RanGTP and cargo is highly cooperative. However, recent crystal structures show that the binding sites for export cargos and RanGTP are located at distant parts of CRM1 [1-3]. We have used a combined approach of all-atom molecular dynamics simulations and small-angle X-ray scattering to study RanGTP and cargo binding to CRM1. We have found that the allosteric effect in CRM1-dependent nuclear export arises from a combination of subtle structural rearrangements and changes in the dynamic properties of CRM1.

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3D-Structural model of Channelrhodopsin-2

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Light-induced phototactic responds in green algae *Chlamydomonas reinhardtii* are mediated via microbial-type rhodopsins, termed Channelrhodopsin-1 (ChR1) and Channelrhodopsin-2 (ChR2)1, which carry the chromophore retinal covalently linked to lysin via a Schiff base and were shown to be directly light-gated ion-channels2. The N-terminal putative seven-transmembrane region of ChR2 was shown to be responsible for the generation of photocurrents and exhibits sequence similarity to the well understood proton pump bacteriorhodopsin (BR) and the sensory rhodopsin *Anabaena* sensory rhodopsin (ASR)3. As for the majority of membrane proteins, there is no 3D-structural data for ChR2 available yet. Here we present homology models of ChR2 using two high-resolution X-ray template structures of BR (1QHJ4) respectively ASR (1XIO5) in order to get structural and functional insights into ChR2. With both homology models we performed molecular dynamics (MD) simulations in a native membrane/solvent environment using GROMACS 4.0.36. Comparison of energetic and structural results revealed obvious advantages of the BR-based homology model of ChR2. Here we show that the BR-based homology model is a reliable model of ChR2 exhibiting structural features already found experimentally7. Our BR-based homology model of ChR2 allows predictions of putative crucial residues within ChR2. So we proposed several mutations within the ChR2 sequence which are already accomplished. Electrophysiologic and spectroscopic studies of these mutations are underway in order to confirm the functional relevance of these residues and to contribute to an optimized usage of ChR2 as a powerful tool in optogenetics.

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Cooperativity in a multidomain protein

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Cooperativity is an important feature in the folding of most proteins. Recently, in a two domain protein such as T4 lysozyme the interdomain cooperativity has been precisely quantified at single molecule level. Such cooperativity has been shown to be dramatically attenuated in a circular permutant which folds to the same native structure. In this paper we exploit this single molecule measurement of cooperativity and a simple molecular model to infer the relationship between the interdomain cooperativity, chain connectivity and free energy landscapes. In particular we show that both species have an onpathway intermediate where one of the domains is completely formed. The change in cooperativity can be ascribed to a change in the rate limiting step for folding. This occurs before the intermediate in the wild type and after in the circular permutant. We suggest that the evolution of discontinuous subdomains may have been facilitated through the exploitation of features of the free energy landscape of an ancestor with continuous subdomains.

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Flash photolysis experiments and molecular dynamics simulation of ligand binding to neuroglobin

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Neuroglobin is a recently discovered globin protein predominantly expressed in brain. Its biological function is still elusive. Despite the fact that neuroglobin shares very little sequence homology to the well- known globins as mioglobin and hemoglobin, they all have a characteristic globin fold with HEME molecule bound to the distal pocket. The structural investigations and CO binding kinetics revealed existence of cavities and tunnels within the protein matrix, where small

ligands can be stored even for hundreds of microseconds [1]. In human neuroglobin there is one internal disulfide bond possible which existence is proved to have significant effect on ligand affinity [2]. In this study effects of temperature, pH, distal histidine mutation and presence of disulfide bond on CO rebinding to neuroglobin are investigated by flash photolysis experiments. In parallel, the molecular dynamics simulations are performed in corresponding conditions in order to investigate structural change of neuroglobin and especially changes in distribution of internal tunnels and cavities able to bind diatomic ligands in response to different physical conditions listed above.

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P-261

Ligand Entry Pathways in PPARγ Explored Using Targeted Molecular Dynamics Simulations

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Computational methods based on molecular dynamics (MD) simulations suite very well for objective exploration of possible ligand entry/exit pathways from protein Ligand Binding Domains (LBDs). Identifying ligand entry/exit routes provides fundamental understanding of protein function and adds substantial information to rational drug design process.
In this area of active research, we focus on PPARs family of nuclear receptors that emerged as therapeutic targets. Agonists have established therapeutics benefits in treating diabetes and cardiovascular diseases.
In this work, we explore possible ligand entry pathways of GW0072, a PPARγ modulator that appears to function as an insulin sensitizer, by using Targeted Molecular Dynamics (TMD) simulations. Starting from the apo-form of PPARγ with GW0072 distal from probable exit gates, TMD simulations converge successfully towards the targeted holo-form of the complex. The simulation results showed that i) entrance paths depend on the protonation state of the ligand; ii) ligand approach suggests that hydrophobic interactions guide the penetration process iii) the routes for ligand access to the *binding* site closely superimpose the most probable ligand exit pathway described in our previous studies (Genest & al. Eur Biophys J 2008).

P-262

Molecular recognition at atomic level: interaction of autism related protein MCP-1 with an antibody

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Autism is often related to infections of autistic children's mothers during pregnancy. The higher activity of the immune system perturbs developing brain and may result in this disorder. It has been found that Monocyte Chemoattractant Protein (MCP-1) levels in brains of autistic children are elevated. Interactions of chemokine MCP-1 with antibodies are important for diagnosis and possible treatment . We present results of classical Molecular Dynamics (MD) studies of MCP-1 complex with a murine Fab antibody fragment [1]. Regions important for molecular recognition (MR) are described and their dynamics is critically analyzed. The process of MCP-1 binding/dissociation is modeled on all-atoms level using the steered MD method by adding a virtual force to increase probability of rare transition in antibody-antigen complexes. The detailed path of MCP-1- antibody MR is analyzed. The interaction mechanism is compared to IgE-grass Phl p2 allergen recognition process studied earlier. These results should help to interpret AFM experiments exploiting lateral force microscopy – a promising technique for MR tracking and medical diagnosis [2].
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P-264

Atomistic simulation of single molecule fluorescence experiments: FRET beyond κ^2=2/3

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Förster Resonance Energy Transfer (FRET) experiments probe molecular distances via distance dependent energy transfer from an excited donor dye to an acceptor dye. Single molecule experiments not only probe average distances, but also distance distributions or even fluctuations, and thus provide a powerful tool to study biomolecular structure and dynamics. However, the measured energy transfer efficiency depends not only on the distance between the dyes, but also on their mutual orientation, which is typically inaccessible to experiments. Thus, assumptions on the orientation distributions and averages are usually made, limiting the accuracy of the distance distributions extracted from FRET experiments. Here, we demonstrate that by combining FRET experiments with the mutual dye orientation statistics obtained from Molecular Dynamics (MD) simulations, improved estimates of distances and distributions are obtained. From the simulated time-dependent mutual orientations, FRET efficiencies are calculated and the full statistics of individual photon absorption, FRET transfer, and photon emission events is obtained from subsequent Monte Carlo (MC) simulations of the FRET transfer kinetics. All recorded emission events are collected to bursts from which efficiency distributions are calculated in close resemblance to the actual FRET experiment, taking shot noise fully into account. Using polyproline chains with attached Alexa 488 and Alexa 594 dyes as a test system, we demonstrate the feasibility of this approach by direct comparison to experimental data. We identified cis-isomers and different static local environments as sources of the

experimentally observed heterogeneity. Reconstructions of distance distributions from experimental data at different levels of theory demonstrate how the respective underlying assumptions and approximations affect the obtained accuracy. Our results show that dye fluctuations obtained from MD simulations, combined with MCsingle photon kinetics, provide a versatile tool to improve the accuracy of distance distributions that can be extracted from measured single molecule FRET efficiencies.

P-265

Molecular Simulations of Thrombospondin's C-Terminal Region

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The thrombospondin family, being extracelluar proteins, is known to be implicated in various physiological processes such as wound healing, inflammation, angiogenesis and neoplasia. The signature domain of thrombospondins shows high sequence identities and thus allows us to transfer results obtained, studying this complex calcium reach part of the proteins, from one member of the family to the other. The domain is known to play a key role in hereditary diseases such as PSACH or MED. In this part of thrombospondins lies a binding site to integrins, important for cell attachment. It is further known that the lectin like globe binds to CD-47, a feature known to be important in cancer research. As the theoretical unit we are trying to resolve these problems by numerical means and are constantly challenged by the size, where thrombospondin can be a huge trimeric protein as one strand can measure 430 kDa, and the large variety of subdomains found in this proteins. We are thus facing a multiscale problem which can range from solving, by means of quantum mechanics a specific ion binding site, to large scale abstraction by continuum mechanics. In our talk we will show you our newest results that we obtained by simulating calcium rich C-terminal domain which is known to be conserved across the entire family, and give you an outlook into the future of our research.

http://upload….28logo%29.svg 6/7/10 2:38 PM Page 1 of 3 http://upload.wikimedia.org/wikipedia/fr/0/0f/ CNRS_fr_quadri.

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P-266

MD-based Modeling of Swift Heavy Ion Beam Nanostructuring of Dielectrics

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The process of swift heavy ions energy deposition while penetrating a solid or scattering on its surface can result in a strong and nonequilibrium excitation of matter. An extremely localized character of this excitation, meanwhile, can make possible both selective changes in chemistry of matter[1] and its surface nanomodifications[2]. Since possible applications have been found in Bio- and IT- technologies (cancer curing and nanostructuring respectively) in the last decade, the heavy ion bombardment technique has attracted a lot of scientific interest[3,4]. The processes of fast energy deposition into the solid and its further dissipation, however, are essentially perturbed with highly excited and nonequilibrium state of both lattice and electron systems. At such conditions therefore, the precision in treatment of processes of electron thermalization, fast electron heat conduction, and phase transformation of the overheated solid becomes crucial. Having several physical models to handle the mentioned processes, it is nevertheless difficult to describe all of them within a scale of a single computational approach. Our work is aimed on elaboration of the atomistic-continuum model[5] of heavy ion bombardment of solids. In particular, the model will be applied to study the formation of nanohillocks in the experiments on swift heavy ion Xe^+ scattering on $SrTiO_3$ surface[2].

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P-267
Transport properties of the human aquaporin HsAQP5
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Aquaporins are protein channels located across the cell membrane with the role of conducting water or other small sugar alcohol molecules (aquaglyceroporins). The presence of the human aquaporin 5 (HsAQP5) in cells proximal to air-interacting surfaces (eyes, lacrimal glands, salivary glands, lungs, stomach etc.) suggest its potentially important role in "wetting" these surfaces. The high-resolution X-ray structure of the HsAQP5 tetramer (PDB code 3D9S) exhibits two important features: (i) lack of the four fold symmetry, common in most of the aquaporins, and (ii) occlusion of the central pore by a phosphatidylserine lipid tail. In this study we investigate the importance of these two features on the transport properties of the human AQP5 by means of molecular dynamics simulations. We found that the asymmetry in the tetramer leads to a distribution of monomeric channel structures characterized by different free energy landscapes felt by the water molecules passing through the channel. Furthermore, the structures' distribution is influenced both by the presence/absence of the lipid tail in the central pore, and by the lipid composition of the bilayer that solvates the HsAQP5 tetramer.

P-268
Elucidating the modular structure of the protein G C2 fragment and human IgG Fc domain binding site using computer simulations
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Protein–protein recognition plays an important role in most biological processes. Although the structures of many protein–protein complexes have been solved in molecular detail, general rules describing affinity and selectivity of protein–protein interactions break down when applied to a larger set of protein-protein complexes with extremely diverse nature of the interfaces.
In this work, we will analyze the non-linear clustering of the residues at the interface between proteins. The boundaries between clusters are defined by clustering the Mutual Information of the protein-protein interface. We will show that the mutations in one module do not affect residues located in a neighboring module by studying the structural and energetic consequences of the mutation. To the contrary, within their module, we will show that the mutations cause complex energetic and structural consequences.
In this study, this is shown on the interaction between protein G C2 fragment and human IgG Fc domain by combining molecular dynamics simulations and mutual information theory, and computational alanine scanning technique. The modular architecture of binding sites, which resembles human engineering design, greatly simplifies the design of new protein interactions and provides a feasible view of how these interactions evolved. The results test our understanding of the dominant contributions to the free energy of protein–protein interactions, can guide experiments aimed at the design of protein interaction inhibitors, and provide a stepping-stone to important applications such as interface redesign.

P-269
Entropy estimates of protein-induced lipid perturbations by molecular dynamics simulations
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Membrane proteins can form large multimeric assemblies in native membranes that are implicated in a wide range of biological processes, from signal transduction to organelle structure. Hydrophobic mismatch and membrane curvature are involved in long range forces largely contributing to such segregation. However, the existing assembly specificity is thought to be coded in the atomic details of protein surface and topology. These are best described in high resolution structures and atomistic molecular dynamics simulations. In order to explore more systematically such forces and energetics arising at intermediate time scales and resolution, we use coarse grained molecular dynamics simulations applied to 20 membrane systems spanning over 5 to 15us. As a first glimpse we study how proteins induce lipid perturbations using a previously developed conformational entropy estimator. We show that in the model membrane where hydrophobic mismatch is present, lipid perturbations extend up to $\sim$40A from the protein surface. However, significant variations in perturbation profiles are seen. Parameters such as protein shape, surface topology, and amino acid physicochemical properties are studied to discover the parameters governing such perturbations.

P-270
Crossing energy barriers with self-guided Langevin dynamics
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Even with modern computer power, the applicability of molecular dynamics simulations is restricted by their ability to sample the conformational space of biomolecules. Often high energy barriers cause normal molecular dynamics

simulations to stay trapped in local energy minima, leading to biased results. To address this problem, Self-guided Langevin dynamics (SGLD) were developed. It enhances conformational transitions by accelerating the slow systematic motions in the system. This is achieved by calculating the the local average of velocities and adding a guiding force along the direction of systematic motions. Thus, the efficiency of SGLD is governed by three factors: a.) the friction constant involved in the Langevin Dynamics b.) the local averaging time and c.) the guiding factor that determines the guiding force. However, the guiding force also causes deviations from the original ensemble that have to be corrected by reweighting the data, thus decreasing the efficiency. Here, we explore the three-dimensional parameter space of SGLD for several benchmark systems with particularly rough energy surfaces. Based on our data, we supply guidelines for the optimal selection of SGLD parameters, to allow the extension of our method to other biological problems of interest.

P-271
Propagation of D816V/H mutation effects across KIT receptor
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Receptor tyrosine kinases (RTKs) regulate critical biological processes. Constitutive activation of RTKs provokes cancers, inflammatory diseases and neuronal disorders. Biological data evidenced that oncogenic mutations of the RTK KIT, located either in the juxtamembrane region (JMR) or in the activation loop (A-loop) - as is the case of D816V/H, displace the equilibrium of conformational states toward the active form. We present a multi-approach study that combines molecular dynamics (MD), normal modes (NM) and pocket detection to characterize and compare the impact of D816V/H on the structure, dynamics and thermodynamics of KIT. We have evidenced a local structural destabilization of A-loop induced by the mutation and a long-range effect on the structure and position of JMR. We have further correlated these observations with experimental data and decipher some details about the activation mechanisms of the mutants, involving leverage of the JMR negative regulation and release of an access to the catalytic site. Through the identification of "local dynamic domains" and the recording of interactions within the protein, we propose a model of the mutational effects propagation, which highlights the importance of both structural distortion and local conformational fluctuations.

P-272
Comparative molecular dynamics study of the stereoisomeric forms of indolicidin and its analogs
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In this study, molecular dynamics (MD) calculations were carried out on the stereoisomeric forms of indolicidin and its analogs with the substitutions of one or more Pro residues by Ala amino acids. According to the *cis-trans* isomerism about the Xaa-Pro peptide bonds, various numbers of stereoisomers could be distinguished for the parent peptide and its Ala-containing derivatives. On the basis of MD simulations, the appearance of different structural properties was investigated, as well as their alterations were studied as a function of time. For each stereoisomeric form of peptides, the presence of secondary structural elements (*i.e.* β-turn and helical structures) and intramolecular interactions (*i.e.* H-bonds, aromatic-aromatic, proline-aromatic and cation-π) was examined. The MD calculations performed on the stereoisomers of indolicidin and its Ala-containing analogs led to the observation that the stereoisomeric forms showed characteristic conformational features. Nevertheless, their appearance proved to be dependent on the *cis* or *trans* nature of Xaa-Pro peptide bonds, as well as on the Pro→Ala substitutions.
This research was supported by the Hungarian Scientific Research Fund (OTKA PD 78554), and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

P-273
Rational design of potential anticancer "drug-like" molecules based on 4-azoldinone scaffold
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Investigation of biological active azolidinones and related heterocycles refer to one of the most successful scientific projects of DH LNMU. It is based on three strategic vectors: organic synthesis, pharmacological research, rational design of "drug-like" molecules (including *in silico* approaches). While applying the research strategy we succeeded in gaining a number of interesting results that make possible to extend the field, especially in the scope of "drug-like" molecules design, notably it has focused on the search of new anticancer agents.
Anticancer activity screening was carried out for more than 1000 compounds (US NCI protocol (DTP) based on obtained directed library over 5000 new compounds, among them 167 compounds showed high activity level. For the purpose of optimization and rational design directions of highly active molecules with optimal "drug-like" characteristics and discovering of possible mechanism of action SAR, COMPARE analysis, molecular docking and QSA(P)R were carried out. Obtained results allowed to form main directions of possible anticancer activity mechanisms, which probable are apoptosis-related. Nowadays the investigation of cellular and molecular aspects of anticancer effects is in progress.

P-274

New insights into the mechanism of mechanical regulation on thiol/disulfide exchange

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Regulation of (Bio)chemical reactions by mechanical force has been proposed to be fundamental to cellular functions[1,2,3]. Atomic force microscopy experiments have identified the effect of mechanical force on the reactivity of thiol/disulfide exchange, a biologically important reaction [4]. In order to understand the influence of the force at an atomistic level, we have performed hybrid quantum mechanics - molecular mechanics (QM/MM) transition path sampling simulation of the reaction under external forces. The results of the simulations supported the experimental findings and demonstrated that the location of the transition state on the free energy surface was shifted due to force [5].
In contrast to our findings, however, a recent experimental study suggests only a weak coupling between the mechanical force and the reaction rate[6]. In this study, the reactants were covalently linked to a stilbene molecule. In this system a force can be applied by photo-isomerization from the relaxed trans to the strained cis configuration. A drawback of this system is that one cannot easily determine the forces that acting on the reaction coordinate. Therefore, we have developed a force distribution analysis method for quantum mechanical molecular dynamics simulations. The results of the analysis show how isomerization of stilbene alters the forces acting on the reacting atoms. The force distribution is essential for understanding how chemistry is controlled by external forces.

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P-275

How lipids affect the side-chain conformational space of a spin label on a protein: an ABF MD study

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Conformational space modelling (CSM) is a promising method for membrane protein structure determination. It is based on the concept of the side-chain conformational space (SCCS), which is formed by the allowed side-chain conformations of a given residue. Each SCCS can be calculated from a 3D structure or measured via EPR-SDSL experiments. For structure determination a set of singly spin-labelled mutants is needed. The final structure is obtained by altering an initial (possibly random) 3D structure until the best fit between the calculated and measured SCCS for the whole set is found.
Such optimization is computationally intensive; therefore CSM includes several empirical approximations. One of them describes the effect of the lipid tails on the SCCS. The implementation is not trivial as lipids diffuse in the plane of the membrane and the lipid tails behave differently at different membrane depths.
To unravel this relationship adaptive biasing force MD simulations were used. An alanine peptide helix was made *in silico*, spin-labelled at the middle and inserted perpendicularly into a DMPC membrane. The free energy of the spin-label orientation at various membrane depths was calculated. A 3D free energy surface describing the membrane "depth" effect was obtained.

P-276

A nano-scavenger in action: the molecular β-cyclodextrin-mediated cholesterol extraction mechanism

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It is known that β-cyclodextrins (βCDs) are able to modify the cholesterol content of cell or model membranes. However the molecular mechanism of this process is still not resolved. Using molecular dynamics simulations, we have been able to study the βCD-mediated cholesterol extraction from cholesterol monolayers and lipid-cholesterol monolayer mixtures. We have investigated many conditions that would affect this process (e.g. lipid-cholesterol ratio, lipid chain unsaturation level)
Our results can be summarized as follow: i) Dimerization of βCDs, ii) binding of the dimers to the membrane surface assuming either a tilted (parallel to the membrane normal) or untilted (90° respect to the normal of the membrane) configuration, iii) the latter configuration is suitable to extract cholesterol at a reasonable computational time scale (100-200 ns), however, this process may be affected by unfavorable energy barriers (from 10 to 80 kJ mol^{-1}), iv) desorption of the complex brings cholesterol in solution, v) the βCD-cholesterol complex is able to transfer cholesterol to other membranes.
With a clearer understanding of the basic molecular mechanism of the βCD mediated process of cholesterol extraction, we can begin to rationalize the design of more efficient βCDs in numerous applications.

P-277
Molecular dynamics simulations of pyrene in POPC and POPC/cholesterol bilayers
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We use molecular dynamics simulations to study bilayers composed of 128 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 120 POPC:30 cholesterol (POPC:Chol 4:1) molecules, with 0, 2 or 4 inserted pyrene molecules. Fluorophore properties such as mass distribution profile along the bilayer normal, orientation, rotational and translational dynamics are monitored, as well as bilayer parameters including area/lipid, mass distribution profiles, translational and rotational dynamics and order parameters. In agreement with recent results reported for fluid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayers, we found that pyrene is preferentially located in the acyl chain region close to the glycerol group of lipid molecules and causes ordering of the lipid acyl chains. On the other hand, and similarly to DPPC gel bilayers (but unlike DPH in DPPC:Chol 4:1), incorporation of pyrene in binary POPC:Chol 4:1 bilayers produces a disordering effect, increasing the area/lipid molecule and decreasing the bilayer thickness and acyl chain order, thus opposing the ordering effect of cholesterol. Funding by FEDER (COMPETE program), and by FCT – Fundação para a Ciência e a Tecnologia, project reference FCOMP-01-0124-FEDER-010787 (FCT PTDC/QUI-QUI/098198/2008) is acknowledged.

P-278
DFT and FTIR-spectroscopic study of polypeptide-ligand complexes
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The mechanism of the complex formation of biopolymers with ligands including the solvent molecules is an actual problem of modern biophysical and biological science. Polypeptides form a secondary structure and mimic the motifs of the protein architecture. The study of complexation between polypeptides and solvent molecules leads to deeper understanding of the basic interaction of proteins with environment at atomic level. Besides polypeptides are promising for the development of applications which encompass some of the following desirable features: anti-fouling, biocompatibility, biodegradability, specific biomolecular sensitivity. On this account polypeptides have a great significance for a variety modern applications ranging from the nanoscale medicine devises up to food technology and others. We compare the results of calculations of complexes between helical polypeptides (polyglutamic acid in neutral form and poly-γ-benzyl-l-glutamate) and water molecules at DFT PBE level and the results of FTIR-spectroscopic study of the film of wetted polypeptides. Vibrational spectroscopy is one of the most useful experimental tools to study non-covalent bonded complexes, and calculated spectra in comparison with experimental data are reliable test for reality of simulated complexes.

P-279
DNA bubbles and bending: how conformational fluctuations modify base pairing
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DNA denaturation is a physical process in the course of which the double strand can open locally thanks to thermal fluctuations. Within a denaturation bubble the two fluctuating single strands have a bending rigidity 50 times weaker than that of the unopened helix, which increase its conformational entropy. The DNA conformation will in turn influence the bubble creation process. This mutual influence naturally leads to a theoretical model coupling the local internal DNA states (open or closed base pairs) and the local DNA elasticity [1-2].
This internal-external coupling allows us to address and answer still open questions, such as how conformational fluctuations modify its thermal denaturation, and why DNA chains are kinked when observed on surfaces by AFM [3].
Finally, we will discuss, by comparing experiments to simulations, the relevance of Tethered Particle Motion experiments to observe molecular events such as denaturation bubble closure in real time [4].

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P-280
Kinetic tools for quantifying platelet aggregation
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Platelet aggregation at the site of vascular injury is vital to prevent bleeding. Excessive platelet function, however, may lead to thrombus formation after surgery. Therefore, an accurate measure and control of platelet aggregation is important. *In vitro* platelet aggregometry monitors aggregate formation in platelet rich plasma triggered by agonists such as ADP, epinephrine or collagen. The fraction of aggregated platelets is plotted versus time, and platelet function is assessed by analyzing the plot's morphology. We propose new measures of platelet function based on a compartmental kinetic model of platelet aggregation induced by ADP. Our model includes three compartments: single, aggregated and deaggregated platelets. It is simpler than earlier models and agrees with experimental data. The

model parameters were determined by non-linear least squares fitting of published data. We associated the kinetic parameters with the activity of the ADP receptors P2Y1 and P2Y12. To this end, we studied published data obtained in the presence and in the absence of specific antagonists of these receptors. Comparison of kinetic parameters of healthy subjects with those of patients with myeloproliferative disorder (MPD) shows that the function of P2Y12 is significantly reduced in MPD.

P-281
Coarse-grained modeling of drug-membrane interactions

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The MARTINI coarse-grained (CG) forcefield was used to simulate the actions of the antimicrobial peptide alamethicin and of the anti-tumoral drug doxorubicin. Both drugs were shown to interact strongly with a fluid phospholipid bilayer, and aggregate there, in agreement with experimental results. Because doxorubicin may establish intermolecular h-bonding, and thus lower its dipole moment, the CG representation of a doxorubicin dimer was adjusted. This less polar dimer was then tested for translocation and/or pore formation.
Contrary to results of atomistic simulations, alamethicin aggregates did not spontaneously open pores. They did so, however, when the size of the water beads was decreased. Several small independent pores could then be observed. The magnitude of the permeability of these pores is analyzed and compared to experimental values. The occurrence of multiple small pores could indicate that the different conductance levels experimentally observed for alamethicin might simply result from the association of different numbers of these small pores.

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P-282
Neuronal (bi)polarity as a self-organized process with membrane growth

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Polarization refers to the asymmetric changes in cellular organization in response to external or internal signals. Neuronal polarization begins with the growth of a single neurite shortly after cell division, followed by the growth of a second neurite at the opposite pole. This early bipolar shape is critical for brain function, as it defines axis of migration and consequently proper three dimensional organization and nerve circuitry. However, it is not known if a direct relationship exists between the formation of the second, opposite, neurite and the mechanisms involved in the formation of the first. We tackled this issue through mathematical modeling, based on membrane traffic (exocytosis-endocytosis), and lateral diffusion. With this approach, we demonstrated that a single pole of molecular asymmetry is sufficient to induce a second one at the opposite side, upon induction of growth from the first pole. Our work gives mathematical proof that the occurrence of a single asymmetry in a round cell is sufficient to warrant morphological bipolarism.

P-283
QM/MM study of serine protease enzyme acylation using DFTB method

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Trypsin is one of the best characterized serine proteases. Enzyme acylation process is required for substrate degradation. There is a lot of information about how this process undergoes. However, in order to obtain a more detailed description of the catalytic triad mechanism, a QM/MM approach was used. We used the hybrid QM/MM potential implemented in AMBER11 package. In the QM calculations a DFT Hamiltonian was used. We develop an approach based on the adaptively biased MD in order to obtain the free energy surface of the conformational space defined by the reaction coordinates. This approach presents some characteristics of steered MD and umbrella sampling procedures. Our results offer information about the lowest energy trajectory, the barrier profile of the reaction, and the geometry of the transition state. This method also provides a further insight into the role of specific residues in the reaction. Substituting Asp102, member of the catalytic triad, for Ala we were able to detect an increase of the barrier profile. This was due to the loss of the interaction of carbonyl group of Asp102 with Nδ of His57, which make Nε of this residue a worst proton acceptor. This results show our approach as a valuable method in the study of enzymatic mechanisms.

P-284
Macromolecular crowding effects studied by THz spectroscopy and molecular modeling

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The intracellular media comprise a great variety of macromolecular species that are not individually concentrated, but being preset in the same compartment they exclude each other's volume and produce crowding. Crowding has a profound impact on protein structure and determines conformational transitions and macromolecular association. We

investigated macromolecular association on a 50% w/w bovine serum albumin (BSA) solution by time-domain terahertz (THz) spectroscopy and molecular modeling. Molecular crowding was simulated by including two BSA molecules in the same water box. We generated ~300 such dimeric models, computed their THz spectra by normal modes analysis and compared them with the experimental data. The best BSA dimer model was selected based on the agreement with the experiment in the lowest frequencies domain of up to 1 THz.

P-285

Symmetry constraints improve accuracy of ion channels models. Application to two-pore-domain channels

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Ion channels are important drug targets. Structural information required for structure-based drug design is often filled by homology models (HM). Making accurate HM is challenging because few templates are available and these often have substantial structural differences. Besides, in molecular dynamics (MD) simulations channels deviate from ideal symmetry and accumulate thermal defects, which complicate HM refinement using MD. We evaluate the ability of symmetry-constrained MD simulations to improve HM accuracy, using an approach conceptually similar to CASP competition: build HM of channels with known structure and evaluate the efficiency of various symmetry-constrained MD methods in improving HMs accuracy (measured as deviation from x-ray structure). Results indicate that unrestrained MD does not improve accuracy, instantaneous symmetrization improves accuracy but not stability during subsequent MD, while gradually imposing symmetry constraints improves both accuracy (by 5–50%) and stability. Moreover, accuracy and stability are strongly correlated, making stability a reliable criterion in predicting HM accuracy. We further used this method to refine HM of TREK channels. We also propose a gating mechanism for mechanosensitive channels that was further experimentally confirmed.

O-286

Nucleotide modifications and tRNA anticodon- mRNA codon interactions on the ribosome

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Molecular dynamics simulations of the tRNA anticodon and mRNA codon have been used to study the effect of the common tRNA modifications cmo^5U34 and m^6A37. In $tRNA^{Val}$ these modifications allow all four nucleotides to be successfully read at the wobble position in a codon. Previous data suggest entropic effects are mainly responsible for the extended reading capabilities but detailed mechanisms have remained unknown. The aim of this paper is to elucidate the details of these mechanisms on an atomic level and quantify their effects. We have applied: extensive free energy perturbation coupled with umbrella sampling, entropic calculations of tRNA (free and bound to the ribosome) and thorough structural analysis of the ribosomal decoding center. The results show that there are three mechanisms responsible for the expanded decoding capability by the modifications: *1. Prestructuring of the ASL decreases entropy.* A decrease of stem loop entropy when free in solution favors the tRNA-ribosome complex formation. *2. Alternate binding conformations.* The further reach of the cmo^5U34 allows an alternative outer conformation to be formed for the non cognate base pairs. *3. Increased contacts between tRNA and mRNA with the ribosome* enhances the "catalyzing" effect of the ribosome.

P-287

Functional and dysfunctional conformers of human neuroserpin: a comparative MD study

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Human neuroserpin (**hNS**) is a serine protease inhibitor (serpin) of a tissue-type plasminogen activator (tPA). The conformational flexibility and the metastable state of this proteins underlies to misfolding and to dysfunctional mutations causing a class of rare genetic diseases which share the same molecular basis. The conformational transition of the **native form**, triggered upon the cleavage at reactive center loop (RLC), releases a complex of the **cleaved form** bound to the inactivated target protease. Without RLC cleavage a stable inactive **latent form** can be obtained by intra/inter molecular loop insertion leading to polymerization. This work concerns the study of the three above mentioned forms of **hNS** by MD simulations to investigate the relation between their conformational stability and. The starting native and cleaved configurations are based on the x-ray structure, while the latent form is here modelled. The results of the simulation reveal a striking conformational stability along with the intrinsic flexibility of selected regions of the fold.The analysis of the essential collective modes of the native hNS shows that the initial opening of the β-sheet A coincides with several changes in the local pattern of salt bridges and of hydrogen bonds.

P-288

Regulation of ubiquitin-conjugating enzymes: a common mechanism based on a pattern of hydrophobic and acidic residues

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E2 ubiquitin-conjugating enzymes play a central role in the ubiquitin (Ub) pathway, influencing the fate of the target substrates [Ye and Rape, Nat Rev Mol Cell Biol 2009,

10:755-64]. Several E2s are characterized by an extended loop (L7), which regulates Ub-charging activity [Papaleo et al, PLoS Comput Biol, in press].
We studied L7 interactions, in representative E2s, integrating phylogenetic and residue co-evolution analyses, normal mode and multi-replica molecular dynamics simulations. A pattern of hydrophobic residues emerged as the main determinant of the stabilization of E2 inactive conformations. L7 can have a pivotal role in downstream events related to interactions with Ub and E3 enzymes. In fact, once the E2-Ub complex is formed, L7 open conformation, induced by phosphorylation, allows its acidic residues to reduce conformational freedom of Ub in the E2 catalytic cleft. Indeed, hydrophobic residues interact with E2 distal regions rich of hydrophobic residues determining a L7 conformation competent for interactions with E3s. In light of the above scenario, L7 emerges as a suitable target for drug design, prompting us to develop virtual screening and docking simulations to identify compounds, which should be able to act as molecular hinges and to modify protein dynamics.

P-289
Protein dynamics and temperature adaptation in extremophilic enzymes
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Enzyme temperature adaptation generally involves a modulation of intramolecular interactions, affecting proteins dynamics, stability and activity [1-2]. In this contribution, we discuss studies of different classes of extremophilic enzymes, focusing on cold-adapted variants, as well as their mesophilic-like mutants, performed by all-atom molecular dynamics simulations with particular attention to structural communication among residues within the three-dimensional architecture [3-4]. Common adaptation strategies turned out to be based on improved local flexibility in the proximity of the functional sites, decrease in interconnected electrostatic interactions, and modulation of correlated motions and networks of communicating residues. Specific differences related to the diverse protein folds can also be detected.
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P-292
Molecular dynamics study of βNeurexin-Neuroligin interactions
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βNeurexins and Neuroligins are cell adhesion molecules and play important role in synapse junction formation, maturation and signal transduction between neurons. Mutations in genes coding these proteins occurs in patients with cognitive diseases like autism disorders, Asperger syndrome and mental retardation [1]. It has been found that the complex βNeurexin-Neuroligin has also an important role in angiogenesis [2]. Herein we will present molecular foundations of βNeurexin-Neuroligin interactions obtained from all-atom molecular dynamics simulations of βNeurexin, Neuroligin and their complex (3B3Q) [3]. 50 ns MD trajectories (CHARMM force field) were analyzed and roles of Ca2+ and N-actetyl-D-glucosamine posttranslational modifications in intermolecular interactions were scrutinized.Supported by Polish Ministry of Education and Science, grant no. N202 262038.

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O-293
The quest for the perfect force field
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Advances in hardware and software have enabled increasingly long atomistic molecular dynamics simulations of biomolecules, allowing the exploration of processes occurring on timescales of hundreds of microseconds to a few milliseconds. Increasing the length of simulations beyond the microsecond time scale has exposed a number of limitations in the accuracy of commonly employed force fields. Such limitations become more severe as the size of the systems investigated and the length of the simulations increase. Here I will describe the force field problems that we have encountered in our studies, how we identified and addressed them, and what we have learned in the process about the biophysics of the systems we are investigating. While the quest for a "perfect" force field is not over (and may never be), our work has greatly improved the accuracy and range of applicability of simple physics-based force fields, to the point that reliable predictions can now be obtained from millisecond-timescale simulations of biomolecules.

P-294

Concentration effects on the encapsulation of prilocaine in liposomes by computer simulations

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Local anesthetics (LA) are pain-relief drugs, widely used in medicine and dentistry. The relatively short duration of analgesia still restricts their clinical use for the treatment of chronic pain. Nowadays, intensive research is focused on anesthetics entrapped into liposomes to enhance their activity and pharmacokinetic properties [1].
In this work, we investigated the encapsulation of prilocaine (PLC), an aminoamide local anesthetic, into a small unilamellar liposome. On the line of our previous work [2], we have carried out Molecular Dynamics (MD) simulations using a Coarse Grain model up the microsecond time scale. In this way, we compare the effects of the concentration of LA at fisiological pH. We were able to capture important features of the PLC-vesicle interactions. The behavior of PLCs at fisiological pH is essentially a combination of high and low pH: we found that all neutral PLC molecules rapidly diffuse into the hydrophobic region of the vesicle adopting an asymmetric bimodal density distribution. Protonated PLC molecules (pPLC) initially placed in water were instead only found on the external monolayer, with a high rate of exchange with the water phase and no access to the inner part of the liposome in a concentration dependent way.

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P-295

Monte Carlo simulation study of diffusion controlled reactions in three dimensional crowded media

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Published data reveal that the rate coefficients of diffusion controlled reactions taking place in crowded media are time dependent. Within this study we have performed on lattice 3D Monte Carlo simulations concerning Michaelis-Menten enzymatic reactions in crowded media, such as the cytoplasmatic region of the cells. We have considered the same size and mobility of the reactant particles and different crowding conditions using distinct concentrations and sizes of the immobile obstacles. The results we have obtained indicate a fractal like kinetics with the degree of fractality that changes with the concentration and dimension of the obstacles. The simulation data also reflect that, depending on the temporal scale, molecular crowding can bring positive or negative effects on reaction kinetics. Comparing the cases studied, for short periods of time the value of the initial rate constant generally increases with the degree of crowding. For longer periods of time and larger distances, molecular crowding slows down the reaction kinetics in every case, due to smaller diffusion coefficients of the reactants, having a more intense effect for the cases with higher degree of crowding.

P-296

Multiscale modeling of adhesion and trafficking

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We focus on applications of molecular and mesoscale simulation methodologies to the cellular transport process of endocytosis, i.e., active transport mechanisms characterized by vesicle nucleation and budding of the cell membrane orchestrated by protein-interaction networks, and functionalized carrier adhesion to cell surfaces. We discuss theoretical and computational methodologies for quantitatively describing how cell-membrane topologies are actively mediated and manipulated by intracellular protein assemblies. We also discuss methods for computing absolute binding free energies for carrier adhesion. We present rigorous validation of our models by comparing to diverse range of experiments.

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P-297

Mg^{2+} is a temporary electron storage during the GTP Hydrolysis Mechanism

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Mg^{2+} is ubiquitous and often coordinated by the triphosphate of GTP or ATP in signal transduction and motor proteins. It

plays an important role in catalytic hydrolysis. Here, we analysed the GTPase Ras which is a crucial switch in cellular signal transduction. It cleaves the substrate GTP hydrolytically to diphosphate and P_i. The GAP protein accelerates GTP hydrolysis by a factor of 10^5. FTIR spectra of GTP show remarkable changes when it binds to the enzyme in comparison to water [1]. The main goal is to understand how this acceleration is accomplished.
Spectral features are computed by QM/MM. The simulations provide charge shifts and small deformations of bond lengths and angles that are not accessible by experiments directly. With GROMACS we calculated 50ns MM trajectories of GTP and GTP bound Mg^{2+} in water, Ras·GTP, Ras·GTP·RasGAP, Ras·GDP·P_i·RasGAP and Ras·GDP. For each system we took six snapshots from the last 25ns as starting structures for 5ps QM/MM simulations, calculated the FTIR spectra and compared them with experiments for validation.
The results give new insights into the role of the Mg^{2+} during hydrolysis: It drags the electrons from the triphosphate, stores them and after the P_i release returns them to the diphosphate.

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P-298
Pathways to exit a receptor: a dynamical view of agonists - delta-opioids interaction
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The importance of delta-opioid receptors as target of a large number of drugs is well recognized, but the molecular details of interaction and action of the compounds are largely unknown. In an effort to shade some light on this important issue we performed an extensive computational study on the interaction of two compounds, clozapine and desmetilclozapine, with a delta-opioid receptor. According to experiments, the lacking of a single methyl group in desmetilclozapine with respect of clozapine makes the former more active than the latter, providing a system well suited for a comparative study. We investigated stable configurations of the two drugs inside the receptor by simulating their escape routes by metadynamics, an algorithm that allows the simulation of events that are otherwise out of range for standard molecular dynamics simulations. Our results point out that the action of the compound is related to the spatial distribution of the affinity sites it visits during its permanency. Desmetilclozapine has a larger distribution of residues, which is interacting with, than clozapine. However, large conformational changes of the receptor were not observed in the presence of both compounds. Thus, a more dynamical picture of ligand-receptor affinity is proposed on the basis of the results obtained, involving the competition among different stable states as well as the interaction with the solvents. Such information might be useful to provide hints and insights that can be exploited in more structure-and-dynamics-oriented drug design.

P-299
Functional modes and residue flexibility control the anisotropic response of Guanylate Kinase to mechanical stress
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Keywords: Protein mechanics, Brownian dynamics, Principal Component Analysis, flexibility, mechanoenzymatics, mechanical anisotropy

The coupling between the mechanical properties of enzymes and their biological activity is a well-established feature that has been the object of numerous experimental and theoretical works. In particular, recent experiments show that enzymatic function can be modulated anisotropically by mechanical stress. We study such phenomena using a method or investigating local flexibility on the residue scale, which combines a reduced protein representation with Brownian dynamics simulations. We performed calculations on the enzyme Guanylate Kinase in order to study its mechanical response when submitted to anisotropic deformations. The resulting modifications of the protein's rigidity profile can be related to the changes in substrate binding affinities that were observed experimentally. Further analysis of the principal components of motion of the trajectories shows how the application of a mechanical constraint on the protein can disrupt its dynamics, thus leading to a decrease of the enzyme's catalytic rate. Eventually, a systematic probe of the protein surface led to the prediction of potential hotspots where the application of an external constraint would produce a large functional response both from the mechanical and dynamical points of view. Such enzyme engineering approaches open the possibility to tune catalytic function by varying selected external forces. (Sacquin-Mora *et al.*, 2010, Biophys. J. **99**, pp3412-3419)

P-300
The initial maturation mechanism of HIV-1 protease
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HIV-1 protease autocatalyses its own release from Gag and GagPol precursor polyproteins into mature functional proteins. As it is functional in the dimeric form, whilst initially, only a single monomer is embedded within each GagPol chain, the question arises as to what cut's the cutter. Two individual monomers in different GagPol chains are known to come together to form an embedded-dimer precursor protease. Mature-like protease activity is concomitant with N-terminal intramolecular cleavage of this transient embedded-dimer precursor, but how this crucial maturation-initiating step is physically achieved, has remained unknown.
Here, we show via 400 all-atom explicit solvent molecular dynamics simulation runs of 400 ns each that the N-terminal

of an immature-like protease, with the N-terminal initially unbound as in the GagPol polyprotein, can self-associate to the active site and therefore be cleaved under conditions of thermodynamic equilibrium, identifying possible binding pathways at atomic resolution, in agreement with previous indirect experimental evidence [1]. The binding pathway predominantly makes use of the open conformation of the beta-hairpin flaps characterised by us previously [2], and the N-terminal binds across the entire active site in good agreement with crystal structures of a cleavage-site peptide-bound protease.
The N-terminus serves two roles, firstly in the maturation of the protease itself by self-associating to the protein and then as a stabilizing component of the dimer interface in a mature protease. Targeting the prior mechanism could be the focus of a novel therapeutic strategy involving immature protease inhibition.

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P-301
Force distribution analysis of allosteric mechanisms
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Revealing the pathways of signal transfer in allosteric proteins has remained a challenge for today's biophysical methods. Previous approaches are primarily based on the comparison of active and inactive structures and thermodynamic concepts. However, stiff regions of the protein might mask signal propagation, even though they are able to carry signals in form of high internal stresses.
We here present a new method, force distribution analysis (FDA Stacklies *et al* 2011, accepted), that detects the distribution of stress upon external perturbations in macromolecules like proteins, other (bio-)polymers or even solids with high sensitivity. For tracing signal transfer through a protein structure, FDA calculates the changes, here caused by ligand binding, in the inter-atomic forces of the protein as sampled in Molecular Dynamics simulations.
The analyzed proteins are two homologues of the chaperone Hsp90 and the catabolite activator protein CAP.
We propose a new model for the signal transduction in the *E.Coli* (HtpG) and Yeast (Hsp82) homologues of Hsp90. The force differences between the apo, ADP and ATP bound states obtained by FDA based on all-atom trajectories totaling 540 ns revealed a cross-talk between the binding site and the middle domain via distinct paths.
The catabolite activator protein (CAP) is a major player in the *lac* operon. CAP features a negative cooperativity for the binding of cAMP, which is interestingly based not on a change in structure but in flexibility (Popovych *et al. 2006)*. FDA of the apo, single- and double-bound state revealed a signaling network in CAP, which transfers a signal (first cAMP is bound) to the second binding niche without obvious structural changes, thereby explaining the observed cooperativity.
This work describes the effectiveness of FDA for resolving allosteric communication pathways, which are directly testable by experiments. As such, it has broad implications for our view on protein internal strain and function.

O-302
The role of non-native interactions in knotted proteins
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Knotted proteins are the object of an increasing number of experimental and theoretical studies, because of their ability to fold reversibly in the same topologically entangled conformation. The topological constraint significantly affects their folding landscape, thus providing new insight and challenges into the funnel folding theory [1]. Recently developed experimental methods to trap and detect knots have suggested that denaturated ensembles of the knotted proteins may be knotted [2].
We present numerical simulations of the early stage of folding of the knotted proteins belonging to protein families MTase (methyltransferase) and SOTCase (succinyl-ornithine transcarbamylase), and of their unknotted homologues [3]. Our results show that native interactions are not sufficient to generate the knot in the denaturated configurations. However, when non-native interactions are included we observe formation of knots only in the chains whose native state is knotted. In addition, we find that the knots are formed through a universal mechanism. Such a knot formation mechanism correctly predicts the fraction of the knotted proteins found in nature and can be used to make qualitative prediction on their folding times.

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P-304
Modeling cellular adhesion with Monte Carlo Simulations
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Cellular adhesion is an important process in cell development and survival, it plays a key role in maintaining cell

shape and motility and also for numerous signaling processes. Adhesion is based on non-covalent interactions between transmembrane proteins and the extracellular matrix. Cells are able to create two-dimensional assemblies of integrins, so called focal adhesions, which they use to stick to the substrate and collect information about the environmental properties. The goal of this work is a deeper understanding of the formation and the stability of these adhesion clusters.
Bond cluster formation and disintegration is dynamically modeled with the aid of Monte Carlo Simulations. In the model, a membrane is attached to a flat surface via a variable number of adhesion bonds. The spacial configuration of these adhesion points subjected to an inhomogeneous stress field maps into a distribution of local membrane/ surface distances. We introduce a model which explicitly accounts for the membrane elasticity and demonstrate that such models are able to explain the spontaneous formation of adhesion bond clusters.

P-305
Characterizeing the activation of protein kinase through an hybrid all-atom structure-based model
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Structure based models are successful at conjugating the essence of the energy landscape theory of protein folding[1] with an easy and efficient implementation. Recently their realm expanded beyond single protein structure, been used profitably to widely study large conformational transitions[2-3]. Still, when dealing with conformational transition between two well-defined structures an unbiased and realistic description of the local backbone and sidechain interactions is necessary. The proposed model merges a precise description of these interactions with a structure-based long range potential that takes into account different conformers. We present the results of the activation of the catalytic domain of human cSRC tyrosine kinase for which we reconstructed the transition free energy and the description of the activation loop flexibility. The excellent model performances in terms of speed and the satisfactory accuracy of the description of the system and its flexibility are promising for a more systematic study of the tyrosine kinase family activation mechanisms.

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P-306
Molecular dynamics simulations on the function of the transmembrane Cav1.2 channel in dependence of the content of cholesterol in the membrane
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Increased cholesterol levels are associated with multiple pathological conditions. Here, Molecular Dynamics simulations are applied to explain the influence of membrane cholesterol levels on voltage-gated calcium channels. We used different lipid compositions to obtain information about the possible effects by which cholesterol influences Cav1.2 channels. Cholesterol is not directly interacting with the protein in the open or the closed conformation, but the Ca^{2+} ion mobility is decreased in POPC/CHOL systems compared to pure POPC bilayers. Cholesterol increases lipid packing implying that it plays a crucial rule in restricting lipid movement in the region around 1 nm of POPC. This leads to an indirect modulation of Cav1.2 channel function.

P-307
Higher order numerical simulation of stochastic chemical reaction systems
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We introduce a previously undescribed technique for modelling the kinetics of stochastic chemical systems. We apply Richardson extrapolation, a sequence acceleration method for ordinary differential equations, to a fixed-step tau-leaping algorithm, to produce an extrapolated tau-leaping method which has weak order of accuracy two. We prove this mathematically for the case of linear propensity functions. We use four numerical examples, two linear and two nonlinear, to show the higher accuracy of our technique in practice. We illustrate this by using plots of absolute error for a fixed-step tau-leap and the extrapolated tau-leap. In all cases, the errors for our method are lower than for a fixed-step tau-leap; in most cases they are second order of accuracy.

O-308

Segment swapping between domains is an evolutionary mechanism that generates new protein folds

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How new protein folds arise during evolution is a long-standing, puzzling question. We present evidence suggesting that new protein structures can arise by a swap of segments between domains within a single-chain monomer. By a comprehensive structural search of the current PDB, we identified 32 well-defined segment-swapped proteins (SSPs) belonging to 18 structural families. Nearly 13% of all multi-domain proteins in the PDB may have a segment-swapped evolutionary precursor as estimated by more permissive searching criteria. The formation of SSPs can be explained by two principal evolutionary mechanisms: (i) domain swapping and fusion (DSF), (ii) circular permutation (CP). By large-scale comparative analyses using structural alignment and HMM methods, it was found that the majority of SSPs have arisen by the DSF mechanism, and a much smaller fraction by CP. Functional analyses revealed that segment swapping, which results in two linkers connecting the domains, may impart directed flexibility to multidomain proteins. Inter-domain segment swapping represents a novel general mechanism by which new protein folds and multi-domain architectures arise in evolution, and segment-swapped proteins have structural and functional properties that make them worth defining as a separate group.

O-310

Molecular dynamics simulations of ribosomal translocation based on cryo-EM data

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High-resolution structural studies of the ribosome have revealed many details of how the ribosomal machinery achieves efficient protein synthesis at the atomic level. These studies provide atomic resolution structures of the ribosome in well- defined states along the protein assembly cycle. Unfortunately, these structures shed little light on intermediate states, transition pathways, and equilibrium states at physiological temperatures. Molecular dynamics simulations allow addressing these dynamical features of the ribosome while maintaining a faithful all-atom, explicit solvent representation of the system. We combine crystallographic and cryo-EM data with molecular dynamics simulations to target the elongation phase of protein synthesis. Using fitted models as starting points we performed molecular dynamics simulations to obtain a picture of the ribosome in different states of transfer RNA translocation. Analyzing the main modes of motion along the simulation trajectories provided insight into the concerted motion of different parts of the ribosome during elongation.

P-311

Implicit solvation in different dielectric environments with the generalized Born model FACTS

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The generalized Born model was introduced for the efficient evaluation of continuum electrostatic energies. FACTS (fast analytical continuum treatment of solvation) is an efficient implicit solvent model based on the generalized Born model for calculating the free energy of solvation of a molecule embedded in an aqueous continuum solvent.
We discuss a modification to the standard FACTS implicit solvent model for modelling heterogeneous dielectric environments with applications to biological membranes. The membrane-water system in the modified model is represented in the form of multiple layered dielectric regions with dielectric constants different from the solute cavity. The modified FACTS model for membranes is implemented in the CHARMM molecular dynamics simulations package and results are shown for a range of different proteins.

P-312

Simulating bacterial efflux: how molecular features affect functional rotation

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The major tripartite efflux pump AcrAB-TolC is responsible for the intrinsic and acquired multidrug resistance in Escherichia coli. At heart of the extrusion machinery there is the homo-trimeric transporter AcrB, which is in charge of the selective binding of structurally and chemically different substrates and energy transduction. The effects of conformational changes, which have been proposed as the key features of the extrusion of drugs, are investigated at molecular level using different computational methods like targeted molecular dynamics. Simulations, including almost half a million atoms, have been used to assess several hypotheses concerning the structure-dynamics-function relationship of the AcrB protein. The results indicate that, upon induction of conformational changes, the substrate detaches from the binding pocket and approaches the gate to the central funnel. In addition, we provide evidence for the proposed peristaltic transport involving a zipper-like closure of the binding pocket, responsible for the displacement of the drug. Using these atomistic simulations the role of specific amino acids during the transitions can be identified, providing an interpretation of site-directed mutagenesis experiments. Additionally, we discuss a possible role of water molecules in the extrusion process.

O-313

Trafficking of lipids between high density lipoprotein and cholesteryl ester transfer protein

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Cardiovascular disease (CVD) is the main cause of death in Western countries. The risk for being exposed to CVD largely depends on the relative levels of low density lipoprotein (LDL) and high density lipoprotein (HDL) particles that are carriers of cholesterol and its esters in human bloodstream. Meanwhile, cholesteryl ester transfer protein (CETP) transports cholesteryl esters, triglycerides, and phospholipids between the different lipoprotein fractions, such as HDL and LDL. Intriguingly, the inhibition of CETP has been shown to prevent and treat the development of CVD. In this work, we employed atomistic and coarse-grained molecular dynamics simulations to unravel the mechanisms associated with the CETP-mediated lipid exchange. We found CETP to bind to the surface of HDL-sized lipid droplets through its charged and tryptophan residues. We further found CETP to induce the formation of a channel from the core of the lipid droplet to the binding pocket of CETP. We also clarified the role of helix X in the opening of CETP binding pocket and the mechanism associated with diffusion of lipids between CETP and the lipid droplet. The findings presented here can be exploited in the design of molecular agents to inhibit the activity of CETP.

P-314

An optimized MM/PBSA virtual screening approach applied on an HIV-1 gp41 fusion peptide inhibitor

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VIRus Inhibitory Peptide (VIRIP), a 20 amino acid peptide, binds to the fusion peptide (FP) of human immunodeficiency virus type 1 (HIV-1) gp41 and blocks viral entry. Molecular dynamics (MD) and molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) free energy calculations were executed to explore the binding interaction between several VIRIP derivatives and gp41 FP. A promising correlation between antiviral activity and simulated binding free energy was established thanks to restriction of the flexibility of the peptides, inclusion of configurational entropy calculations and the use of multiple internal dielectric constants for the MM/PBSA calculations depending on the amino acids sequence. Bases on these results, a virtual screening experiment was carried out to design enhanced VIRIP analogues. A selection of peptides was tested for inhibitory activity and several improved VIRIP derivatives were identified. These results demonstrate that computational modelling strategies using an improved MM/PBSA methodology can be used for the simulation of peptide complexes. As such, we were able to obtain enhanced HIV-1 entry inhibitor peptides via an MM/PBSA based virtual screening approach.

P-317

Structure based discovery and optimization of first-in-class inhibitors of the HIV-1 IN–LEDGF/p75 interaction

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An essential step during the HIV life cycle is the integration of the viral cDNA into the human genome. HIV-1 integrase mediates integration in a tight complex with the cellular cofactor: LEDGF/p75 [1]. Disruption of the interaction interferes with HIV replication and therefore provides an interesting new drug target for antiretroviral therapy [2,3]. Here we present the structure based discovery and optimization of a series of small molecule inhibitors that bind to HIV-1 integrase and block the interaction with LEDGF/p75. The work flow was set up according to a funnel principle in which a series of virtual screening tools were applied in such way to discard at each step molecules unlikely to be active against the desired target (including 2D filtering, pharmacophore modelling and molecular docking) The activity and selectivity of the selected molecules were confirmed in an ALPHA screen based assay, that measure protein-protein interaction in vitro, and furthermore by in vivo experiments. Active compounds proceeded towards crystallographic soaking into the receptor protein crystals. These crystal structures not only validated the binding mode and activity of the hit compounds, but furthermore were used as a platform for structure based drug design which resulted in the rational discovery of new hit compounds and optimized lead compounds. *In vitro* and *in vivo* experiments validated the mechanism of action of these compounds and show that they are a novel class of antiretroviral compounds with in vivo inhibitory activity by targeting the interaction between LEDGF/p75 and HIV-1 integrase. Cross resistance profiling indicate that these compounds are active against current resistant viral strains.[4] Currently the most potent inhibitors show an *in vivo* IC_{50} of 55nM. These compounds are promising for future pharmaceutical optimizations to be used in the clinic as new antiretroviral agents.

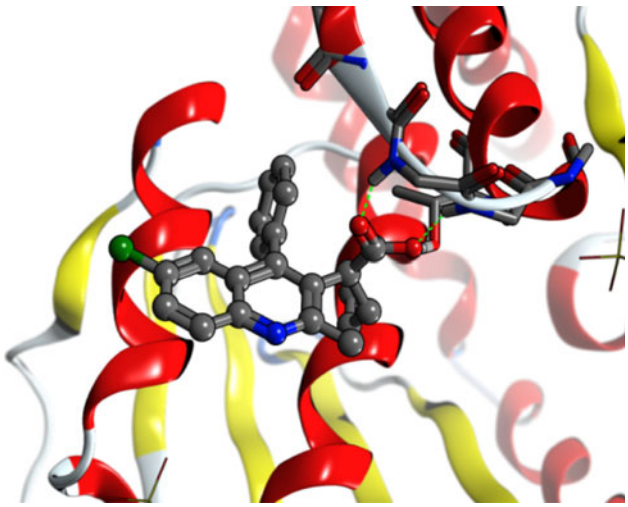

Figure: Crystallography was used to validate the binding mode of the discovered inhibitors. Insights in the interaction of the ligand-protein complex allowed for rational design of optimized inhibitors.

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P-318

Modelling allosteric signalling in protein homodimers

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Allostery in protein systems is a thermodynamic phenomenon. Allosteric response is driven by the free energy differences obtained in different binding events, which, in principle, contain contributions from both enthalpic and entropic changes. While traditional views of allostery have concentrated on structural changes induced by the binding of ligands (i.e. "enthalpically dominated"), it is now increasingly recognised that fluctuations in structure can contribute to allosteric regulation. In some cases, where ligand-induced structural changes are small, thermal fluctuations can play a dominant role in determining allosteric signalling. In thermodynamic terms, the entropy change for subsequent binding is influenced by global vibrational modes being either damped or activated by an initial binding event. One advantage of such a mechanism is the possibility for long range allosteric signalling. Here, changes to slow internal motion can be harnessed to provide signalling across long distances. This paper considers homotropic allostery in homodimeric proteins, and presents results from a theoretical approach designed to understand the mechanisms responsible for both cooperativity and anti-cooperativity. Theoretical results are presented for the binding of cAMP to the catabolite activator protein (CAP) [1], where it is shown that coupling strength within a dimer is of key importance in determining the nature of the allosteric response. Results from theory are presented along side both atomistic simulations and simple coarse-grained models, designed to show how fluctuations can play a key role in allosteric signalling in homodimeric proteins.

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P-319

A forward-backward mathematical model of blood pressure and blood flow in human upper limb

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Keywords: Pulse wave reflection, blood pressure waveforms, arterial hemodynamics

A forward-backward mathematical model of the blood flow and the blood pressure of the large arteries in human upper limb have been developed. The model is based on Womersley solution for one-dimensional equations derived from the axisymmetric Navier-Stokes equations for flow in a vessel. Blood flow was measured at brachial and radial artery using IR sensor, positioned at 3 cm distance from arterial branching point, where the pulse was easily detected by hand. The arterial pulse wave velocity, reflected coefficient and terminal impedance were calculated from the measured data reflected coefficient and used in simulation. Blood flow and blood pressure simulations were compared with the measured signals as well as with the published clinical data. The model predicts the blood pressure waveform composed of a forward propagation waveform and a number of reflected waveforms from many branching sites. The model predictions are in a good correlation of with the age-related characteristics of blood vessels. The model is consistent with clinically observed blood pressure and can be useful for proper biophysical understanding of arterial hemodynamics.

Imaging and optical microscopy

P-320

A new class of reversibly switchable fluorescent proteins

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Reversibly switchable fluorescent proteins (RSFPs) can be switched between a fluorescent (on) and a nonfluorescent (off) state which is accompanied by a cis-trans isomerization of the chromophore on the molecular level 1,2. This unique property has already provided new aspects to various microscopy techniques, such as high resolution microscopy, FCS or monochromatic multicolor microscopy 3-5. Despite of their established potential, RSFPs still have a major drawback: the wavelength for fluorescence excitation is always one of the two switching wavelengths. The imaging process thus inevitably results in the switching of a small fraction of the RSFPs, which might hinder or complicate some experiments. We developed a new reversibly switchable fluorescent protein which eliminates the problem of the coupling between switching and fluorescence excitation. This fluorescent protein follows an unusual and currently unknown mechanism of switching between a fluorescent and a non-fluorescent state. It is brightly fluorescent and exhibits an excellent signal to noise ratio.

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O-321

Programmable array microscope imaging of living cells and spin-off to tumor diagnostics

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EGFR exhibits diffusion but undergoes retrograde transport to the cell body after ligand association, dimerization, and transautophosphorylation. Filopodial transport allows remote sensing and was discovered by sensitive imaging of QD-EGF [1]. Recently we identified Shc1 as an essential component for coupling of activated EGFR to the actin filaments [Gralle Botelho et al.]
In parallel studies [2], QD-based ligands (EGF, Mabs) were targeted to EGFR in gliomas. Cell-cultures, animal models and *ex vivo* biopsies of human high-grade as well as low-grade gliomas showed high probe specificity.. The aim is to define more precisely the tumor boundaries at the time of resection. We used the **P**rogrammable **A**rray **M**icroscope designed for sensitive, high-speed optical sectioning, particularly of living cells. The PAM is based on structured illumination and conjugate detection using a digital micromirror device (DMD) [3] located in a primary image plane. The unique feature is the rapid, (re)programmable adjustment of local excitation intensity, Dynamic, on-the-fly optimization is thus achieved, e.g. multipoint FRAP [4], light exposure minimization and object tracking [5], or super-resolution strategies. The features and operation of the 3rd generation PAM will be presented.

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O-322

Three-dimensional video-rate nonlinear microscopy of contracting myocytes

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Contraction of muscle cells, motility of microorganisms, neuronal activity, and other fast cellular processes require microscopic imaging of a three-dimensional (3D) volume with a video-rate scanning. We present 3D video-rate investigations of structural dynamics in biological samples with the multicontrast third- and second-harmonic generation as well as fluorescence microscope. The multidepth scanning is achieved by two combined laser beams with staggered femtosecond pulses. Each of the beams is equipped with a pair of deformable mirrors for dynamic wavefront manipulation enabling multidepth refocusing with simultaneous corrections for optical aberrations. Combined, more than 250 frames per second lateral scanning with fast refocusing enables the 3D video-rate imaging of dynamically moving structures. In addition, combination of two laser beams is accomplished at two perpendicular polarizations enabling live imaging of sample anisotropy, which is important for structural studies particularly with the second harmonic generation microscopy. Investigations of beating chick embryo hearts with the 3D video-rate scanning microscope revealed multidirectional cardiomyocyte contraction dynamics in myocardial tissue. Intricate synchronization of contractions between different layers of myocytes in the tissue will be presented. The video-rate 3D microscopy opens new possibilities of imaging fast biological processes in living organisms.

P-323

Spinning-disk confocal fluorescence microspectroscopy: instrumental setup and features

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Confocal fluorescence microscopy is an invaluable tool to study biological systems at cellular level also thanks to the synthesis of always new specific fluorescent probes, *e.g.* multiprobe labelling enables complex system characterization. However, only the recent employment of narrowband tunable filters overcomes the problems due to the use of the broadband ones. The possibility of acquiring the emission spectra in a spatially resolved way extends simple image intensity studies into characterization of complex probe-environment relationship through the sensitivity of fluorescence spectra to the local molecular environment differences. Consequently, fluorescence microspectroscopy (FMS) is able to provide the spectral information in a well defined spatial region allowing the researcher to simultaneously obtain spatial and spectroscopic information. Our instrument has been specially built to study live cells and their interaction with nanomaterials, drug carriers and modified cell environment. Other main characteristics are reducing the bleaching effect and employing a white light source that does not limit the use to specific probes. Graphical tools, such as colour coded images, have also been introduced to provide explicit and straightforward visual information.

P-325

High speed FPGA based multi-tau correlation for single-photon avalanche diode arrays

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With the evolving technology in CMOS integration, high-density arrays of single-photon avalanche diodes (SPAD) have become available. We use a 32x32 SPAD pixel array as image sensor in a custom built Single Plane Illumination Microscope for Fluorescence Correlation Spectroscopy (SPIM-FCS). Our array can be read out at rates above 100kHz for full frames, thus enabling observation of fast biophysical and -chemical processes at the microsecond timescale. The time-dependent fluorescence fluctuations are evaluated by means of an autocorrelation analysis for every single pixel. To cover a wide range of lag-times, the multiple-tau correlation algorithm is used. We implemented this algorithm in a Field Programmable Gate Arrays (FPGA), a reconfigurable integrated circuit. Due to their parallelism these devices are ideally suited for such algorithms. Our current hardware design is capable of calculating full autocorrelation of all 1024 pixels in real-time for a given frame-time of 10μs. The same design can also be used for cross-correlation. This enables us to observe diffusion processes of the samples in real-time.

P-326

FRET-Imaging to reveal cross-bridge dynamics in single skeletal muscle fibres

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We demonstrate the use of FRET-imaging (Forster Resonance Energy Transfer) as an assay to directly monitor the dynamics of cross-bridge conformational changes in single fibres of skeletal muscle. We measured nm-distances of several FRET pairs located at strategic positions to sense myosin head conformational changes: we focused our attention on the essential light chain, ELC (specifically labelling a modified ELC and exchanging it with the natural ELC of the fibre) and we investigated its interaction with the SH1 helix, with the nucleotide binding pocket and with actin. We characterized FRET in single rigor muscle fibres, determining distances in agreement with those from the crystallographic data. The results demonstrate the viability of the approach in sensing different FRET efficiencies over a few nanometres, an essential requirement to follow the expected small FRET variations in contracting muscle fibres. We are now performing dynamic experiments on rigor and active fibres by applying small stretch/release cycles to alter the interaction distances (estimated time resolution of nearly 20ms/frame). In this configuration, it will be possible to measure functional changes, shedding light on the myosin head dynamics during contraction.

P-327

Direct visualization of CFTR conformation by atomic force microscopy imaging

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Cystic Fibrosis (CF) is one of the recessive Mendelian genetic diseases more popular in the world. There is no decisive treatment for this disease and, only recently, an effort to find pharmacological therapies for CF has been carried out. This disease is caused by a mutation in the gene encoding for the CF trans-membrane regulator (CFTR) chloride channel present in epithelial cells. A full knowledge of the CFTR structure and conformational changes associated with the activation process would allow a better understanding of the disease, possibly leading to the identification of suitable drugs. Up to now a detailed molecular structure of the first nucleotide binding domain (NBD1) was determined by x-ray diffraction and an homology model of the NBD1-NBD2 dimer was build and successively extended to the complete CFTR. However, the

quaternary organization of the protein is still controversial: X-ray experiments suggest that isolated CFTR is organized as monomer, whereas a dimeric organization is proposed on the basis of electron microscopy reconstructions. In addition, Schillers et al. (2004) used Atomic Force Microscopy (AFM) to image the cytoplasmic side of CFTR on transfected Oocytes, showing the presence of peculiar annular structures, probably associated to CFTR oligomers, but never observing features comparable with the proposed structures for monomers and dimers. The aim of our work is to study CFTR in its natural environment, the plasma membrane, to gather definite information about the native organization of the channel and to open for future studies on the putative functional role of the supramolecular organization of the channel in the plasma membrane that has not yet been fully addressed. Atomic Force Microscopy (AFM) imaging was performed on the cytoplasmic side of cellular membranes extracted from fetal rat thyroid (FRT) cells permanently transfected with CFTR. The protocol to obtain flat and clean membranes, crucial to reach the highest resolution in AFM images, was carefully assessed by means of AFM and confocal microscopy. CFTR channels were identified in the plasma membrane using an immunogold labeling approach and the organization of their quaternary structure was studied. Our preliminary results indicate that several CFTR conformations co-exist in the plasma membrane: monomers, dimers and annular rings.

P-328
Focal delivery of BDNF to cultured neurons with optical tweezers

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Focal stimulation of cultured neurons is crucial since it mimics physiological molecules release. Indeed, the nervous system finely tunes the activity of each synapse by regulating the secretion of molecules spatially and temporally. Currently used techniques have some drawbacks such as a poor spatial resolution or a low flexibility. We propose a novel approach based on Optical Tweezers (OT) [1] to overcome these limitations.
OT allow an ease manipulation with sub-micrometric precision of silica beads, which can be functionalized with any protein. For a proof-of-principle study we coated 1,5μm large beads with Brain-Derived Neurotrophic Factor (BDNF) or Bovine Serum Album (BSA) as control. We showed that a single bead was able to activate the BDNF receptor TrkB, inducing its phosphorylation. Moreover BDNF beads but not control beads were able to induce c-Fos translocation into the nucleus [2], indicating that the whole pathway was activated. Finally, we positioned vectors in proximity to the growth cones of cultured hippocampal neurons [3]. Control beads didn't affect the normal development of these structures while BDNF beads significantly did. These findings support the use of the OT technology for long-term, localized stimulation of specific subcellular neuronal compartments.

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O-329
Optical nanoscopy and individual molecule localization with focused light under linear and non linear regimes

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A recognized advantage of optical microscopy lies in the fact that allows non-invasive three-dimensional (3D) imaging of live cells at the submicron scale with high specificity. The advent of the visible fluorescent proteins and of a myriad of fluorescent tags pushed fluorescence microscopy to become the most popular imaging tool in cell biology. The confocal and multiphoton versions of fluorescence microscopy reinforce this condition. In general, is a well-known paradigm the given inability of a lens-based optical microscope to discern details that are closer together than half of the wavelength of light. Recently, the viewpoint for improving resolution moved from optical solutions to the side of the fluorescent molecule to be detected. Today, for the most popular imaging mode in optical microscopy, i.e. fluorescence, the diffraction barrier is crumbling and the term "optical nanoscopy", coined earlier, comes to be a real far field optical microscope available for the scientific community as the ones allowing individual molecule localization at high precision. Here we discuss about architectures, calibrations and applications of targeted and stochastic readout methods using both single and multiphoton excitation.
Work supported by IIT, IFOM and Italian PRIN 2008JZ4MLB.

P-330
Fluorescence resonance energy transfer analysis of nuclear proteins by laser scanning cytometry

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Defining location and nature of the interactions between molecular species in living cells is hampered by the limited resolution of the employed instruments. We present here a slide–based cytometric method of fluorescence resonance energy transfer analysis that utilizes spectral properties of cyan and yellow fluorescent proteins as markers to investigate the interaction of Fos and Jun transcription factors in

substratum attached cells. FRET was measured by a laser scanning imaging cytometer which combines the advantages of flow cytometry with those of the image analysis system. Large number cells was automatically identified, their fluorescence data and digital images were determined on cell by cell basis. FRET efficiencies of CFP and YFP tagged Fos-Jun heterocluster pairs was 8.35±3.00% representing 6.98nm proximity of Fos-Jun protein terminals. The background FRET efficiency due to spectral crosstalk in the absence of molecular interaction was 0.42±0.64%. On population level the applied transfection method provides FRET events with low efficiency, FRET signals from Fos-Jun protein pairs, however, were clearly located in the nuclei of the cells.

P-331

Chromosome imaging: fluorescence in situ hybridisation (FISH) in human peripheral blood lymphocytes

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Fluorescence in situ hybridisation (FISH) relies on the hybridisation of labelled DNA probes in a complementary way and allows direct visualization of chromosome region of interest. Study aimed to describe FISH technique application in human peripheral blood lymphocytes for further use in radiobiological investigations. The whole chromosome 2XCP probe for chromosome 2 (MetaSystems, Germany), 6q21/ Alphasatellite Cocktail probe for chromosome 21 telomeric and centromer region (Q-biogene) and Telomere PNA Probe/ Cy3 (Dako) for telomeric regions were applied on lymphocytes slides. Samples were counterstained with DAPI + Antifade solution. Localisations of above mentioned specific probes were analyzed with Olympus fluorescent microscope and ISIS software (MetaSystems, Germany) on 100 methaphases for each samples using DAPI and FITC filters. Cells with good signals for each of applied probes were scored. It was showed that 2XCP probe for chromosome 2 and Telomere PNA Probe/Cy3 for telomeric regions worked very efficiently and could be recommended for further analysis. Most of the cells hybridised with 6q21/Alphasatellite Cocktail probe had splited signal, especially in case of green signal (centromer region) and eventual modification of protocols for this type of cells may be proposed.

P-332

Visualization of subcellular localization of pro/anti - apoptotic factors after Hypericin induced photodynamic treatment

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Hypericin (Hyp) is a highly hydrophobic plant pigment with photosensitizing properties. Hyp localization inside cell plays a key role of its photoactivity, due to the singlet oxygen production, which has a very short lifetime (ns-μs, depending of Hyp environment). Hyp sub-cellular localization depends on its concentration in the medium, incubation time and used delivery system.
Variations in activity of Protein Kinase C, (anti-apoptotic PKCα and pro-apoptotic PKCδ) in correlation with the activity of Bcl-2 protein, cytochrome c release from mitochondria and decreasing of mitochondrial membrane potential after photodynamic action were monitored. The study was performed for two different delivery modes of Hyp to U- 87MG glioma cells- Hyp alone (membrane diffusion) vs. Hyp loaded in low density lipoprotein (LDL) (endocytosis). Confocal fluorescence microscopy, flow-cytometry and specific fluorescence labeling were used as main experimental techniques. Our results show that Hyp photoaction strongly affects apoptotic response of the cells and that the dynamics of this action significantly depends on used delivery system. Correlation analysis between the monitored parameters (see above) determined for both delivery system is presented and critically discussed.

Acknowledgements
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P-333

Correlative dynamic optical microscopy (TL, FRAP, FCS, FLIM) to decipher medical biofilm reactivity

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Surface contamination by bacteria is a natural and spontaneous process occurring in any natural environment on biotic (mucosa, tissues…) and abiotic surfaces (medical equipments, food surfaces…). Whatever the bacterial nature (Gram-positive or -negative), the environmental fluid (air, water, blood…) and the receptor surface (implants, medical equipments, food surfaces…), the surface contamination initiated by the first adherent bacteria can evolve to a three dimensional structure named biofilm (cohesive bacteria assembly ensured by an autoproduced extracellular organic matrix). The mechanisms by which these biofilms offer protective environment to viral particles or hypertolerance to antimicrobial action are not yet elucidated. To reach a better understanding of biofilm reactivity, we reported for the first time successful applications of correlative time-resolved optical microscopy approach by time-lapse (TL), FRAP, FCS,

FLIM, for real-time analysis of molecular mobility and reaction inside biofilms. By means of non-biological or biological (virus, biocides and antibiotics) reactive compounds, significant advances to understand the roles of the extracellular matrix and bacteria physiological properties were obtained, an important step to improve pathogenic biofilm inactivation.

P-334
Towards skin cancer diagnostics by two-photon microscopy
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Here we present a feasibility study to develop two-photon microscopy (2PM) into a standard diagnostic tool for non-invasive, skin cancer diagnosis. The goal is defining experimental parameters to maximize image quality of optical biopsies in human skin, while avoiding tissue damage. Possible diagnostic indicators will be compared for healthy tissue, benign, and malignant melanocytic lesions.
We report on preliminary results of a study on 2PM imaging of "ex-vivo" biopsy samples, were autofluorescence intensity and contrast between lesion and surrounding tissues were optimised varying excitation wavelength, detection band, and dispersion pre-compensation. Moreover, we determined modulation functions for laser power and detector gain to compensate losses in deep tissue imaging. As the main process of photo-damage, thermo-mechanical modifications were quantified and damage threshold powers were determined.
In order to image structural changes in ordered tissue like collagen fibres, second-harmonic generation signals were recorded and optimised.

P-335
In-vivo two-photon imaging of the honeybee antennal lobe
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We adapted a two-photon microscope for in-vivo imaging of the honeybee olfactory system, focusing on its primary centres, the antennal lobes. The setup allowed obtaining both 3D-tomographic measurements of the antennal lobe morphology and time-resolved in-vivo calcium imaging of its neuronal activity.
The morphological data were used to precisely measure the glomerular volume in both sides of the brain, investigating the question of volumetric lateralization. Functional calcium imaging allowed to record the characteristic glomerular response maps to external odour stimuli applied to the bees' antennae. Compared to previous neural imaging experiments in the honeybee, this work enhanced spatial and temporal resolution, penetration depth, and it minimized photo-damage. Final goal of this study is the extension of the existing functional atlases of the antennal lobe to 3D and into the temporal dimension by investigating the time-resolved activity pattern.

P-336
Fluorescent analysis of apoptosis induced by *Pseudomonas aeruginosa* in endothelial cells
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A consequence of invasion by an intracellular pathogen is apoptosis of the host cell, usually observed in phagocytic cells. The aim of this study was to evaluate if *Pseudomonas (P.) aeruginosa,* although not considered a classic intracellular pathogen, could induce apoptosis in endothelial cells (EC). Strains were evaluated for virulence factors expression: pore forming toxins (haemolysins, lecithinases, lipases), proteases (caseinases, gelatinasse), DNA-ses, mucinases. The adherence and invasion capacity of *P. aeruginosa* to EC was done using Cravioto's method. For detection of apoptosis was used acridine orange/ethidium bromide staining. The qualitative assay of bacterial adherence to cellular substrate reveled that all tested strains adhered to EC, exhibiting a diffuse, aggregative or mixed diffuse-aggregative pattern and 20-70% adherence rates. Cell free culture of *P. aeruginosa* did not determine a cell death response, but in case of whole bacterial culture, acridine orange/ethidium bromide staining showed the capacity to induce apoptosis of EC, fluorescent microscopy revealed the chromatin condensation, fragmented nuclei, membrane blebbing, and apoptotic bodies. *P. aeruginosa* have the ability to adhere and invaded EC and to induce morphological changes, including apoptosis.

P-337
Photo-toxicity of near-infrared voltage sensitive fluorescent dyes
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The use of voltage sensitive fluorescent dyes (VSD) for noninvasive measurement of the action potential (AP) in

blood perfused heart have been hindered by low interrogation depth, high absorption and auto-fluorescence of cardiac tissue. These limitations are diminished using new near infrared (NIR) VSD (di-4-ANBDQBS). Here we validated toxicity and photo-toxicity of these dyes in guinea pig and human cardiac muscle slabs.

Application of NIR VSD showed no effect on cardiac muscle contraction force or relaxation. Optical action potentials closely tracked kinetics of microelectrode recorded APs in both field and electrode stimulated preparations. For photo-toxicity assessment dye (50 μM) preloaded cardiac slabs were exposed to prolonged laser radiation of various power. Microelectrode AP recordings show that exposure to prolonged laser radiation (10min.; 2mW/mm^2) of dye loaded tissue had no statistically significant effect on APD50 or conduction velocity, thus indicating no or weak photo-toxicity on the NIR VSD. In contrast, exposure to 5 min. laser radiation of phototoxic dyes (mitotracker deep-red) preloaded tissue caused significant reduction in APD50 (by 13%) and conduction velocity (30%). Thus, due to the low photo-toxicity, NIR VSD are well suited for in vivo cardiac imaging.

P-338

Morphological analysis of spores with various microscopic techniques in *Streptomyces coelicolor*

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Streptomycesetes are filamentous Gram-positive soil bacteria well known for their complex morphological development and secondary metabolite production. During their life cycle spores germinate to form a network of hyphae, which later develops into aerial mycelium when cross-walls are generated and spores are formed. We have examined and compared the last stage of the differentiation process in a wild-type *S. coelicolor* (M145) and its *ΔcabB* mutant lacking a calmodulin-like calcium binding protein.

The strains were grown on four kinds of media: SMMS, SMMS with 10 % saccharose, R5 and R5 with reduced calcium in order to study the effect of environment and osmotic stress on the sporulation of the two strains to assess the function of CabB protein.

From the cultures pictures were taken at 48 hours and after 7 days using phase contrast, atomic force and confocal laser scanning microscope and the sizes of spores were measured.

Our results showed that the *ΔcabB* mutant made smaller spores, its differentiation and stress response were slower. We could conclude from it that the aberrant protein slows the metabolism, the signal transduction and has effects on sporulation, septation and air-mycelium formation. Based on it we can tell that the CabB has a significant role in normal development.

P-339

Implementation of dual auto- and cross correlation function calculation for FCS/FCCS measurements by FPGA

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Fluorescence correlation and crosscorrelation spectroscopy have become widely used techniques for studying the diffusion and interactions of molecules. From a combination of autocorrelation (ACF) with dual color crosscorrelation functions (XCF) a more complex picture can be gained about the diffusing species and their interactions. Most hardware correlator cards lack the capacity to calculate auto- and crosscorrelation functions and store raw data simultaneously. Here we present a new approach to calculate ACFs and XCFs online simultaneously by using the multiple tau and multiple sampling time algorithm implemented on a field programmable gate array (FPGA, National Instruments). Although the FPGA has strict source restriction, the number of available correlation functions can be multiplied by using this new approach. Thus the dual ACF and dual XCF can be calculated for continuous illumination. For calculation of the desired correlation functions a series of interconnected linear correlator blocks were implemented. The first 16 correlator blocks had a minimal lag time of 50 ns, followed by 23 blocks of 8 correlators with stepwise doubled sampling times. The new approach is based on the interleaved scheduling of the execution of linear correlator blocks.

P-340

Single plane illumination fluorescence correlation spectroscopy using high speed image sensors

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The mobility and reaction parameters of molecules inside living cells can be conveniently measured using fluorescent probes. Typically fluorescence correlation spectroscopy (FCS) based on confocal microscopy is used for such measurements. This implies high time-resolution but only for a single spot at a time. In order to achieve a high time-resolution at multiple spots, we built a single plane illumination microscope (SPIM) equipped with high-speed image acquisition devices and a high-NA detection optics. This allows us to do parallel FCS measurements in a thin plane (width ~ 2-3 μm) of the sample. Our setup is equipped with a fast EMCCD camera (full frame time resolution 2000μs) and a 32×32 pixel array of SPADs. The SPAD array has a full frame time resolution of 3-10μs, which is even fast enough to

resolve the typical motion time-scale of small molecules (like eGFP) inside living cells. The performance of the system is characterized by diffusion measurements of water-soluble fluorescent beads, as well as FCS measurements in living cells. Our data acquisition system uses programmable hardware for some tasks and is fast enough to allow real-time correlation of 1024 pixels, as well as saving the complete dataset for later evaluation.

O-341

Cryo-EM of membranes and membrane proteins

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Electron cryo-microscopy (cryo-EM) covers a larger size range than any other technique in structural biology, from atomic resolution structures of membrane proteins, to large non-crystalline single molecules, entire organelles or even cells.
Electron crystallography of two-dimensional (2D) crystals makes it possible to examine membrane proteins in the quasi-native environment of a lipid bilayer at high to moderately high resolution (1.8-8Å). Recently, we have used electron crystallography to investigate functionally important conformational changes in membrane transport proteins such as the sodium/proton antiporters NhaA and NhaP, or the structure of channelrhodopsin.
"Single particle" cryo-EM is well suited to study the structure of large macromolecular assemblies in the 3.3 to 20Å resolution range. A recent example is our 19Å map of a mitochondrial respiratory chain supercomplex consisting of one copy of complex 1, two copies of complex III, and one of complex IV. The fit of the x-ray structures to our map indicates short pathways for efficient electron shuttling between complex I and III by ubiquinol, and between complex III and IV by cytochrome *c*.
Electron cryo-tomography can visualize large protein complexes in their cellular context at 30-50Å resolution, and thus bridges the gap between protein crystallography and light microscopy. Cryo-ET is particularly suitable for studying biological membranes and large membrane protein complexes *in situ*. We found that long rows of ATP synthase dimers along ridges of inner membrane cristae are an ubiquitous feature of mitochondria from all 6 species we investigated (2 mammals, 3 fungi, 1 plant). The proton pumps of the respiratory chain appear to be confined to the flat membrane regions on either side of the ridges. This highly conserved pattern suggests a fundamental role of the mitochondrial cristae as proton traps for efficient ATP synthesis.

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Modeling the red blood cell shapes for holographic imaging

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Digital holographic microscopy is a label free, noncontact, nonintrusive and nondestructive technique which gives quantitative information about phase shift introduced by the transparent samples. From one single hologram, without mechanical scanning in the experimental setup, we reconstruct the amplitude and the phase, which allow 3D imaging of the sample with nanometer accuracy along propagation axis. On the CCD camera, we record the diffraction pattern of the sample, superposed with the reference beam. The study is focused on investigating the iso-intensity curves in the diffraction pattern coming from the phase-only transmission functions which contain oblate spheroid models for the red blood cell (RBC) shapes. In simulations, the values interval for dimensions and refractive indices are in concordance with the real ones, in different situations. The experimentally diffraction patterns in given conditions are obtained using spatial light modulator. Results obtained in Fresnel approximation will be used as preprocessing observations in our microfluidic applications. Results obtained in Fraunhofer approximation can be used in ektacytometry.
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O-343

Rational design and applications of photoconvertible and bi-photochromic fluorescent proteins

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Advanced fluorescence imaging, including subdiffraction microscopy, relies on fluorophores with controllable emission properties. Chief among these fluorophores are the on/off photoswitchable and green-to-red photoconvertible

fluorescent proteins. IrisFP was recently reported as the first fluorescent protein to combine irreversible photoconversion from a green-emitting to a red-emitting state with reversible on/off photoswitching in both the green and red states. The introduction of this protein resulted in new applications such as super-resolution pulse-chase imaging, but the properties of IrisFP are far from being optimal from a spectroscopic point of view and its tetrameric organization complicates its use as a fusion tag. We have demonstrated how four-state optical highlighting can be rationally introduced into photoconvertible fluorescent proteins by developing and characterizing a new set of such enhanced optical highlighters derived from mEosFP and Dendra2. One of these, which we called NijiFP, was identified as a promising new multi-photoactivatable fluorescent protein with optical properties that make it ideal for advanced fluorescence-based imaging applications.

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Synthesis and optical properties of ultrasmall inorganic optical markers based on lanthanides emission for biomedical applications

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Introducing to medicine and biology concept of optical markers in tremendous way has changed the recent status of these two important disciplines. This was mainly due to strong development in imaging techniques which recently allow us to investigate both static as well dynamic properties of living cells, their components and their interactions with external factors.
Recently used molecular markers including organic dyes, fluorescent proteins or chelates containing lanthanide ions have several significant limitations. One of the alternatives for molecular markers are inorganic quantum dots (ie. CdSe, CdS) which are recently commonly used in many academic works. However, even if they are much better from physicochemical point of view, from the application point of view at this moment they are rather useless mainly because of their high risk of toxicity. One of the solution combining advantages of both concepts is to make nontoxic inorganic nanocrystals doped by lanthanide ions.
In this work, we will present optical results obtained for $NaYF_4$ nanocrystals doped by different lanthanide ions. The aim of this work was to design and to synthesize these markers and to understand physical processes responsible for their emission and to optimize these processes to the physical limits.

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Natural killer cells fate at the draining lymph nodes: a physical portrait of the biological contest

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Intravital microscopy has fostered a full blown of publication regarding the behavior of cells in different tissues and physiological conditions. However, a few papers describe how the motility parameters can be used to understand whether an interaction is occurring and, on balance, the distinction between interacting and non interacting cells is performed visually on the image time stack.
Here we describe a multi-parameter approach that allows to discern among different cell behaviors on an objective ground and we demonstrate its effectiveness valuating the mutual fate of Natural Killer (NK) and Dendritic (DC) cells at the draining lymph-nodes in inflammatory and stationary conditions.
The method is time saving and allows a wide scale characterization of the lymphocyte tracks and to build up statistics of the cell-cell interaction duration. This has allowed the development of a numerical model of the NK-DC interaction, based on a molecular-stochastic dynamic approach, whose output can be directly compared to the data. The Smoluchowsky equation is resolved for two superimposed radial potentials irradiating from the DC cells and describing the short and long range DC-NK interaction mediated by different types of interleukins.

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Time-resolved Raman imaging of malarial hemozoin

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Hemozoin is formed during malaria infection of red blood cells, the malaria parasite cleaves hemoglobin, leaving free heme which is then toxic to the parasite. The free heme is then bio-crystalized to form hemozoin which allows the parasite to remain viable. The hemozoin is released during the breakdown of the red blood cells, is small and can be difficult to resolve spatially. Since it contains an abundance of heme protein, which has a strong absorbance at 532 nm, it can be readily detected and tracked by using resonant Raman scattering spectroscopy. Here we use slit-scanning confocal Raman imaging to detect the hemozoin and resolve it against the background molecules. Inside a red blood cell, hemoglobin is the strongest background signal since it also contains large amounts of heme. Nevertheless, the discrimination is possible, and the time-resolved observation of hemozoin is an important tool to understand effects of malaria since the hemozoin can trigger the immune response and cause inflammation in tissue.

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Superresolution optical microscopy of isolated cardiac mitochondrial proteins

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To study the structural organization of mitochondrial proteins, we applied Stimulated Emission Depletion (STED)

microscopy in isolated mitochondria. In STED microscopy, two laser beams are used: one for excitation of fluorophores and the other, with doughnut shape, to deplete them in order to allow fluorescence emission only from the excited volume located at the doughnut's center. Using a custom-made STED, we achieved a lateral resolution of ~20 nm in images of purified mitochondria, where we investigated the localization pattern and distribution of Cox 4 and VDAC1 proteins, and confirmed the existence of Na/Ca^{2+} exchanger. Results show that Cox 4 proteins are distributed in clusters of ~20 and ~28 nm; whereas VDAC1 displays four size distributions of ~22, ~33, ~55 and ~83 nm. We have demonstrated that protein clusters in the mitochondria can be resolved with a separation power of ~20 nm, and that it is possible to retrieve quantitative information about their cluster sizes. This approach can be extended to other proteins in mitochondria and subcellular organelles and to investigate disease-induce changes in mitochondrial protein distribution. Supported by NIH and AHA.

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Integrin-dependent activation of the JNK signaling pathway in live cells by mechanical stress

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Mechanical force is known to modulate the activity of the Jun N-terminal kinase (JNK) signaling cascade. However, it is not known how this activation is regulated *in vivo*. We assessed in real-time the activity of the JNK pathway in *Drosophila* S2R+ cells by Fluorescence Lifetime Imaging Microscopy (FLIM) using an intramolecular phosphorylation-dependent dJun-FRET (Fluorescence Resonance Energy Transfer) biosensor. We found that *Drosophila* S2R+ cells plated on different substrates showed distinct levels of JNK signaling that associate with differences in cell morphology, stiffness, and integrin and focal adhesion organization. Quantitative FRET-FLIM analysis also revealed the sustained activation of the JNK pathway in response to multidirectional static stretch. Importantly, this was contingent on the presence of functional integrins in focal adhesions but not on Talin. In contrast, at rest, both integrins and Talin suppressed JNK activation. Our results showing sustained JNK activation and stable cell morphology changes in response to mechanical stretch suggest that alterations in the cytoskeleton and matrix attachments may act as regulators of JNK signaling and that this property itself might feed back to modulate cell adhesion and the cytoskeleton. This dynamic system is highly plastic; at rest focal adhesions and Talin are key factors in modulating JNK activity, while mechanical stretch leads to a Talin-independent JNK activation. The level of JNK activity seems to be a major element, but not the only one, in the regulation of the cytoskeleton and cell shape remodeling associated with stretch. Finally, in combination with RNA silencing experiments we have been able to dissect the signal transduction pathway for mechanical stress in this system.

O-350

Direct mapping of nanoscale compositional connectivity on intact cell membranes

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Lateral segregation of cell membranes is accepted as a main mechanism for cells to regulate a diversity of cellular functions. In this context, lipid rafts have been conceptualized as organizing principle of biological membranes where underlying cholesterol-mediated selective connectivity must exist at the resting state. However, such a level of nanoscale compositional connectivity has been challenging to prove. We used single-molecule near-field scanning optical microscopy (NSOM) to visualize the nanolandscape of raft ganglioside GM1 after tightening by its ligand cholera toxin (CTxB) on intact cell membranes. We show that CTxB tightening of GM1 is sufficient to initiate a minimal raft coalescence unit, resulting in the formation of cholesterol-dependent GM1 nanodomains <120nm in size. Simultaneous dual color high-resolution images revealed that GPI anchored proteins were recruited to regions proximal (<150nm) to CTxB-GM1 nanodomains without physical intermixing. Together with *in-silico* experiments, our high-resolution data conclusively demonstrate the existence of raft-based interconnectivity at the nanoscale. Such a linked state on resting cell membranes constitutes thus an obligatory step towards the hierarchical evolution of large-scale raft coalescence upon cell activation.

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X-ray spectro-microscopy at ID21: mapping of elements and chemical states at subcellular level

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Microscopy techniques at sub-micron resolution are key tools for research in life sciences, and one of their most desirable features is elemental sensitivity. The beamline ID21 of the

European Synchrotron Radiation Facility [1] hosts a Scanning X-ray Microscope (SXM) for X-ray fluorescence spectromicroscopy: its relatively wide energy range (2-9.2 keV) and the small X-ray beam size (0.3x0.7 μm^2, achieved by means of Zone Plate or Kirk-Patrick Baez mirror focusing) allow to image elemental distributions at cellular and sub-cellular resolution, with sensitivity to trace elements down to a few ppm. Furthermore, the chemical state of a selected element can be determined by means of micro-X-ray-Absorption Spectroscopy (μXAS).
Molecular mapping with $\sim$5μm lateral resolution can as well be achieved on ID21, thanks to a Synchrotron-Radiation (SR) FTIR-microscope: the combination of SXM and SR-FTIR provides a set of complementary information necessary to investigate the physics and chemistry of biosystems.
The experimental capabilities of the beamline and its versatility (wide variety of sample environments and detection systems) will be presented, together with examples of applications, notably to stem cells research.

[1] M.Salomé et al (2009), J. Phys.: Conf. Ser. 186, 012014

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Inside the polyribosome by super-resolution microscopies
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During translation initiation, the 5' cap and the 3' poly(A) tail of a eukaryotic mRNA are recognized by eIF4E and PABP (poly(A)-binding protein), respectively. Both yeast and human eIF4E interact with eIF4G, eIF4A and eIF4B forming the eIF4F complex. This 5' cap complex cooperates with the poly(A) tail to promote a functional circularization of mRNA, which is necessary for efficient translation by bridging of its 5' and 3' termini by the protein-protein interaction. Despite biochemical and genetic evidences demonstrate the interaction of eIF4G and Pab1, up to now the imaging information on these proteins and their relationship with the mRNA, the native closed-loop and the organization of the UTRs in the polysome are completely lacking. Here, we focus on these fundamental topics by integrating three different high resolution imaging approaches by comparing images of native human polysomes obtained by Atomic Force Microscopy, cryo-EM and STED microscopy.

P-353
Optical signal interrogation depth in Tyrode and blood-perfused myocardial tissue
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Photon attenuation length (δ) in biological tissues determines interrogation depth, spatial resolution, and amplitude of fluorescence signal in various types of optical imaging, including imaging of cardiac excitation using voltage-sensitive dyes. We assessed δ in human and pig myocardium at excitation/emission wavelengths of commonly used and recently developed near-infrared voltage-sensitive dyes (NIR). We also compare δ in Tyrode vs blood-perfused tissues and simulate respective fluorescent signals in the context of potential clinical applications.
Experiments were conducted in isolated slabs of ventricular myocardium. Light decay inside the tissue was measured at 520, 650, and 715nm. δ in blood-perfused porcine and human myocardium were similar (at 520nm 0.46$\pm$0.01 mm and 0.48$\pm$0.02 mm; at 650nm 1.83 $\pm$ 0.02 mm and 1.98 $\pm$ 0.07 mm, at 715nm 2.31 $\pm$ 0.05 mm and 2.74 $\pm$ 0.13 mm in porcine and human respectively), which makes porcine myocardium a good model for development of clinical imaging applications. Blood- vs Tyrode perfusion significantly reduces δ, particularly at 520nm. However, our simulations show that, the resulting reduction of optical action potential is $<$16% (for NIR dyes), which would not be a major impediment for in vivo imaging of cardiac excitation.

Molecular motors

O-354
Myosin on an optical leash: load dependence of the working stroke in a single myosin head
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Muscle performance at the molecular level is determined by the elementary displacement (working stroke) produced by the motor protein myosin II, and its dependence on load. We developed a laser trap assay (the optical leash) capable of applying controlled loads to a single myosin head before the working stroke is initiated and probing actin-myosin interaction on the microsecond time scale. We found that the working stroke size depends both on load and on the detachment pathway followed by myosin. In a first pathway, myosin detaches very rapidly from actin ($<$1ms) without producing any movement. In a second pathway, myosin steps and remains bound to actin for a time inversely

proportional to ATP concentration; the working stroke remains constant ($\sim$5 nm) as the load is increased, until it suddenly vanishes as the isometric force is reached (5.7$\pm$0.6pN). A third dissociation pathway becomes more populated as the force is increased, due to premature unbinding of myosin from actin, resulting in a working stroke that decreases with load. Taken together, these results give a new insight into the molecular mechanism of load dependence of the myosin working stroke, which is a primary determinant of skeletal muscle performance and efficiency.

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Mechanism of DNA sequence recognition by the SpoIIIE translocase

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During cell division, chromosomal DNA must be segregated rapidly and accurately. In bacteria, chromosomal segregation is conducted by DNA translocases, *e.g.* FtsK in *Escherichia coli* or SpoIIIE in *Bacillus subtilis*. Sporulating *B. subtilis* divides asymmetrically. To assure the genetic material integrity after sporulation, SpoIIIE assembles at the division septum and translocate the 2/3 of the chromosome ($\sim$3 Mbp) remaining in the mother cell towards the forespore by using the energy of ATP hydrolysis converted into mechanical force by their AAA+ motor domains. Several mechanisms have been proposed in order to understand the determinants of translocation directionality of DNA translocases. It is accepted that direction of translocation is signed by specific and asymmetrical 8 base pair chromosomal sequences, but how these enzymes recognize this specific sequences and what is their role in activity and directionality regulation still generates controversy. Here, we combine information from SpoIIIE/DNA equilibrium and pre-equilibrium binding assays with continuous triplex displacement measurements to further elucidate the mechanism of SpoIIIE sequence recognition and its effect on transport directionality.

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Rotation of F_1-ATPase with a protrusion-less gamma rotor

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F_1-ATPase is an ATP-driven rotary motor in which the central γ subunit rotates inside a stator made of an $\alpha_3\beta_3$ ring. ATP hydrolysis in the β subunits generates torque for γ rotation by a mechanism not fully understood. The γ rotor consists of a globular domain that protrudes from the stator and a slender shaft that penetrates the $\alpha_3\beta_3$ ring. The shaft is an anti-parallel coiled coil of the N- and C- terminal helices of γ. Previously we have deleted either or both of these terminal helices genetically. Surprisingly, all mutants rotated in the correct direction, showing that the shaft portion is dispensable. Here we inquire if the rest of the γ rotor, the globular protrusion that accounts for $\sim$70 % of the γ residues, is also dispensable. Keeping the N- and C-terminal helices that constitute the shaft, we have replaced the middle $\sim$200 residues with a short helix-turn-helix motif borrowed from a different protein. The protrusion-less mutant thus made retained a high ATPase activity and rotated fast in the correct direction. This may not be unexpected because, in crystal structures, most of the removed residues do not contact with the $\alpha_3\beta_3$ ring. Combined with the previous results, however, the present results indicate that none of the γ residues are needed for rotation.

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The rotary mechanism of a molecular engine, the vacuolar proton-ATPase, working in a biomembrane

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The rotary mechanism of vacuolar proton-ATPase (V-ATPase) couples ATP hydrolysis and trans-membrane proton translocation. We tested the effect of oscillating electric (AC) field on V-ATPase activity in yeast vacuoles. The AC technique has several advantages over direct observations: it can be applied on native membranes, there are no labels and attachments involved, and the target protein is in its natural environment. This was/is the first of its kind of experiment on V-ATPase, and we got strikingly different results from previous studies on other proteins: Both low and high frequency AC field reduces ATPase activity in a wide frequency range. A sharp resonance is seen at 88.3 Hz, where the ATPase activity reaches or exceeds the control (no AC) level. We think that the resonance happens at that of the 60 degrees rotor steps, meaning that the rotation rate of the rotor is around 15 Hz, under the given conditions. Synchronisation of individual ATPases by slow or matching, but not fast AC is likely via a hold-and-release mechanism. We can explain the above observations by assuming that the AC field interacts with the proton movements, and if we consider the estimated geometry of the hydrophilic proton channels and the proton binding cites on the rotor.

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The effect of stretch on phosphate release in skeletal muscle fibres

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We applied ramp stretches to contracting permeabilized fibres of mammalian skeletal muscle at 12 and 20°C, with a view to better understand the relationship between force and the elementary steps of the actomyosin ATPase. Using a fluorescent, genetically engineered phosphate binding protein to report on phosphate (Pi) release resulting from ATP hydrolysis, we determined the time course of Pi release during 1) development of isometric force, 2) a ramp stretch applied when the isometric force plateau has been reached, 3) an isometric phase after stretch and 4) reversal of the stretch. The results show that the rate of Pi release rapidly decreases upon the application of stretch, with observable changes in Pi occurring as quickly as those observed in the force signal. At the end of the stretch, force decreases whilst the rate of Pi release accelerates. During ramp shortening, force decreases to the level expected from the force-velocity relation, and the rate of Pi release accelerates as ATP hydrolysis fuels the exertion of power by the muscle fibre. The rapid decrease in the rate of Pi release upon stretch is explained by a redistribution of cross-bridge states to enhance Pi-containing intermediate states at the expense of intermediate states containing ADP only.

P-359

Stepping rotation in H^+-ATPase/synthase has been revealed with an essentially drag-free probe

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Vacuole-type ATPases (V_oV_1) and F_oF_1 ATP synthases constitute a superfamily of rotary molecular machines that couple ATP hydrolysis/synthesis in the soluble V_1/F_1 portion with proton flow in the membrane-embedded V_o/F_o portion, by sharing one joint rotary shaft. Here we have compared at submillisecond resolutions the ATP-driven rotation of isolated V_1 and the whole V_oV_1 derived from *Thermus thermophilus*, using a 40-nm gold bead for which viscous drag is almost negligible. At saturating ATP of 4 mM, V_1 rotated at $\sim$60 revolutions per second, with $\sim$5 ms dwells every 120°. Dwell time analyses indicated that at least two events other than ATP binding, one likely ATP hydrolysis, occur in each dwell, as in F_1. Unlike F_1, however, the dwells were at ATP-waiting positions that were resolved at μM ATP. V_oV_1 rotated an order of magnitude slower, and exhibited dwells separated by $\sim$30°. The twelve positions, though not always fully populated, match the twelve-fold symmetry of the V_o rotor in *T. thermophilus*, indicating that the ATP-driven rotation must go through stator-rotor interactions in V_o, possibly coupled with proton translocation. We plan to examine the pH dependence of the dwells to see if they are indeed coupled to protonation/deprotonation.

O-360

How myosin traps and how actin triggers the release of phosphate at the beginning of force generation

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Coupling structural and kinetic studies has elucidated details of a subset of the transitions of the actin-myosin catalytic cycle, including the ATP-induced dissociation of myosin from actin and for the ATP hydrolysis step that allows priming of the lever arm. Not yet visualized however are the structural rearrangements triggered by actin binding that are coupled to force generation and product release. While phosphate is produced in the pre-powerstroke (ADP.Pi) state, it is also trapped in this state until an actin-activated transition opens a back-door to promote its release from the active site. It is unclear, however, which of the three elements of the active site (Switch I, Switch II and the P-loop) play a role in this transition and whether the major cleft within the motor partially closes to allow this release while keeping the lever arm primed. New structures of the myosin VI motor in the pre-powerstroke state with Pi trapped reveal the post-hydrolysis state that traps the motor. We have also crystallized a new structural state for myosin VI in the presence of MgADP and phosphate that has an open back-door due to a Switch II rearrangement and a lever arm primed. Thus it has the expected features of the previously unseen Pi release state of myosin. Kinetic and structural studies on a number of mutants from different myosins are currently being performed to evaluate this possibility. The structure is consistent with cleft closure occurring subsequent to Pi release, which is also being tested. An interesting finding in this new structure is that the SH1 helix kinks to allow the converter to stay in the primed position, the signature of a state at the beginning of force production.

O-361

Assembly and function of the type 3 secretion system needle from *Salmonella typhimurium*

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Pathogenic Gram-negative bacteria including *Salmonella typhimurium*, *Shigella flexneri* and *Yersinia pestis* use a Type Three Secretion System (TTSS) to deliver virulence factors into host cells[1] [2]. The TTSS constitutes a continuous protein transport channel of constant length through the bacterial envelope[3]. The needle of the Type Three Secretion System is made of a single small protein (protomer). We analyzed the assembly and the structure of the TTSS needle using different biophysical methods including Fourier Transform-Infrared Spectroscopy, nmR spectroscopy and X-ray crystallography. We show that the TTSS needle protomer refolds spontaneously to extend the needle from the distal end. The protomer refolds from α-helix into β-strand conformation to form the TTSS needle[4]. Regulated secretion of virulence factors requires the presence of additional protein

at the TTSS needle tip. X-ray crystal structure analysis of the tip complex revealed major conformational changes in both the needle and the tip proteins during assembly of the *S. typhimurium* TTSS. Our structural analysis provides the first detailed insight into both the open state of the TTSS needle tip and the conformational changes occurring at the pathogen-host interface[5].

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Binding change mechanism of membrane-bound F_o rotary motor driven by proton motive force

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The membrane-bound component F_o of the ATP synthase works as a rotary motor and plays the central role of driving the F_1 component to transform chemi-osmotic energy into ATP synthesis. We have conducted molecular dynamics simulations of the membrane-bound F_o sector with explicit lipid bilayer, in which the particular interest was to observe the onset of helix motion in the *c* ring upon the change of the protonation state of Asp61 of the c_1 subunit, which is the essential element of the Boyer's binding-change mechanism. To investigate the influence of transmembrane potential and pH gradient, i.e., the *proton motive force*, on the structure and dynamics of the *a-c* complex, different electric fields have been applied along the membrane normal. Correlation map analysis indicated that the correlated motions of residues in the interface of the *a-c* complex were significantly reduced by external electric fields. The deuterium order parameter (S_{CD}) profile calculated by averaging all the lipids in the F_o-bound bilayer was not very different from that of the pure bilayer system, which agrees with recent ^{2}H solid-state nmR experiments. However, by delineating the lipid properties according to their vicinity to F_o, we found that the S_{CD} profiles of different lipid shells are prominently different. Lipids close to F_o formed a more ordered structure. Similarly, the lateral diffusion of lipids on the membrane surface also followed a shell-dependent behavior. The lipids in the proximity of F_o exhibited very significantly reduced diffusional motion. The numerical value of S_{CD} was anti-correlated with that of the diffusion coefficient, i.e., the more ordered lipid structures led to slower lipid diffusion. Our findings will not only help for elucidating the dynamics of F_o depending on the protonation states and electric fields, but may also shed some light to the interactions between the motor F_o and its surrounding lipids under physiological condition, which could help to rationalize its extraordinary energy conversion efficiency. This work has been published[1] in March 2010, and it was selected as one of the two Featured Articles of that issue.

1. Lin, Y.-S.; Lin, J.-H.; Chang, C.-C., Molecular Dynamics Simulations of the Rotary Motor F0 under External Electric Fields across the Membrane. Biophys. J. 2010, 98, 1009-1017.

P-363

Neck linker docking coordinates the kinetics of kinesin's heads

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Conventional kinesin is a two-headed motor protein, which is able to walk along microtubules processively by hydrolyzing ATP. Its neck linkers, which connect the two motor domains and can undergo a docking/undocking transition, are widely believed to play the key role in the coordination of the chemical cycles of the two motor domains and, consequently, in force production and directional stepping. Although many experiments, often complemented with partial kinetic modeling of specific pathways, support this idea, the ultimate test of the viability of this hypothesis requires the construction of a complete kinetic model. Considering the two neck linkers as entropic springs that are allowed to dock to their head domains and incorporating only the few most relevant kinetic and structural properties of the individual heads, we have developed the first detailed, thermodynamically consistent model of kinesin that can (i) explain the cooperation of the heads (including their gating mechanisms) during walking and (ii) reproduce much of the available experimental data (speed, dwell time distribution, randomness, processivity, hydrolysis rate, etc.) under a wide range of conditions (nucleotide concentrations, loading force, neck linker length and composition, etc.). Besides revealing the mechanism by which kinesin operates, our model also makes it possible to look into the experimentally inaccessible details of the mechanochemical cycle and predict how certain changes in the protein affect its motion.

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Toward the construction of a myosin based machine

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The research project is directed toward the construction of a synthetic bio-inorganic machine that consists in a single

actin filament that interacts with a linear array of myosin II motors regularly disposed on a nano-structured device. The motor array is intended to simulate the unique properties of the ensemble of motor proteins in the half-sarcomere of the muscle by providing the condition for developing steady force and shortening by cyclic interactions with the actin filament. The mechanical outputs in the range 0.5-200 pN force and 1-10,000 nm shortening will be measured and controlled with millisecond time resolution by means of a double laser optical tweezers apparatus (DLOT, Bongini et al., *Biophys J, Supplement 1*, **98**:752a/3908-Pos, 2010). The objectives pursued by the project are: a. Production of native and artificial myosins; b. Interfacing motor proteins and inorganic devices; c. Preparation of bead tailed actin with correct polarity; d. Design and fabrication of nano-structures to control the geometry of the motor protein array by nano-patterning of the silica/quartz support; e. Synthetic machine imaging by optical and electron microscopies. The advancement of the research in each of these objectives is reported.

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Mechanism of the force generation in the bacterial flagellar motor: a structural biology perspective

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The bacterial flagellar motor is a membrane-embedded molecular machine that rotates filaments, providing a propulsive force for bacteria to swim. The molecular mechanism of torque (turning force) generation is being investigated through the study of the properties and three-dimensional structure of the motor's stator unit. We are taking both top-down and bottom-up approaches, combining data from the molecular genetics studies, cross-linking, X-ray protein crystallography and molecular dynamics simulations. We have recently determined the first crystal structure of the protein domain that anchors the proton-motive-force-generating mechanism of the flagellar motor to the cell wall, and formulated a model of how the stator attaches to peptidoglycan. The work presented at the meeting will inform the audience on our latest work that establishes the relationship between the structure, dynamics and function of a key component of the bacterial flagellar motor, the motility protein B (MotB). This work will be put in the perspective of the mechanism of rotation, stator assembly, anchoring to peptidoglycan and interaction with the rotor, and discussed in the light of the elementary events composing the cycle of electrochemical-to-mechanical energy conversion that drives flagellar rotation.

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Directionality of the yeast Kinesin-5 Cin8 is controlled by molecular structure and salt conditions

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Members of the conserved Kinesin-5 family fulfill essential roles in mitotic spindle morphogenesis and dynamics and were thought to be slow, processive microtubule (MT)-plus-end directed motors. The mechanisms that regulate Kinesin-5 function are still not well understood. We have examined *in vitro* and *in vivo* functions of the *Saccharomyces cerevisiae* Kinesin-5 Cin8 using single-molecule motility assays and single-molecule fluorescence microscopy and found that Cin8 motility is exceptional in the Kinesin-5 family.
In vitro, individual Cin8 motors could be switched by ionic conditions from rapid (up to 50 μm/min) and processive minus-end, to bidirectional, to slow plus-end motion. Deletion of the uniquely large insert of 99 amino acids in loop 8 of Cin8 induced bias towards minus-end motility and strongly affected the directional switching of Cin8 both *in vivo* and *in vitro*. We further found that deletion of the functionally overlapping Kinesin-5 Kip1 and of the spindle-organizing protein Ase1 affected Cin8 velocity and processivity, but directionality was not affected.
The entirely unexpected finding of switching of Cin8 directionality *in vivo* and *in vitro* demonstrates that the "gear box" of kinesins is much more complex and versatile than thought.

O-368

Longitudinal and rotational motion of microtubules driven by the kinesin-14 motor Ncd

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In gliding motility assays, various motor proteins have been shown to drive not only translational, but also rotational motion of filaments around their longitudinal axis. Using FLIC microscopy [1], we measured the longitudinal and rotational motion of quantum-dot labeled microtubules driven by Ncd (a kinesin-14 motor protein) as a function of the ATP concentration. The data show two striking features: (i) The longitudinal velocity shows a strong deviation from Michaelis-Menten shape. (ii) The rotational pitch depends strongly on the ATP concentration. We propose a simple mechanical model, which explains both findings. The underlying mechanism of the non-processive Ncd motor assumes that the power stroke comprises a longitudinal, as well as a lateral

(off-axis) component and that the waiting (apo) state takes place before the power stroke. Our model is distinct from the models for filament rotation by myosin kinesin-1 motors, which is a consequence of the filament structure [2, 1]. Our results support the finding that the Ncd power stroke is triggered by ATP binding [3].

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O-369
Switch between large hand-over-hand and small inchworm-like steps in myosin VI
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Many biological motor molecules move within cells using stepsizes predictable from their structures. Myosin-VI, however, has much larger and more broadly-distributed stepsizes than those predicted from its short lever arms. We explain the discrepancy by monitoring Qdots and gold nano-particles attached to the myosin VI motor domains using high-sensitivity nano-imaging. The large stepsizes were attributed to an extended and relatively rigid lever arm; their variability to two stepsizes, one large (72 nm) and one small (44 nm). These results suggest there exist two tilt-angles during myosin-VI stepping, which correspond to the pre- and post-power-strokes states and regulate the leading head. The large steps are consistent with the previously reported hand-over-hand mechanism, while the small steps follow an inchworm-like mechanism and increase in frequency with ADP. Switching between these two mechanisms in a strain sensitive, ADP-dependent manner allows myosin-VI to fulfill its multiple cellular tasks including vesicle transport and membrane anchoring. http://www.fbs.osaka-u.ac.jp/labs/yanagida/, http://www.qbic.riken.jp/.

Structure of proteins and protein complexes

P-370
Mössbauer spectroscopy of chicken liver and spleen, human liver ferritin and pharmaceutical models
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Ferritin deposits iron in oxyhydroxide iron core surrounded by protein shell. The iron core structure may vary in different ferritins in both normal and pathological cases. To study iron core variations the Mössbauer spectroscopy with a high velocity resolution was applied for comparative analysis of normal and leukemia chicken liver and spleen tissues, human liver ferritin and commercial pharmaceutical products Imferon, Maltofer® and Ferrum Lek as ferritin models. Mössbauer spectra of these samples measured with a high velocity resolution at room temperature were fitted using two models: homogenous iron core (one quadrupole doublet) and heterogeneous iron core (several quadrupole doublets). The results of both fits demonstrated small variations of Mössbauer hyperfine parameters related to structural variations of the iron cores. These small structural variations may be a result of different degree of crystallinity, the way of iron package, nonequivalent iron position, etc. Obtained small differences for normal and leukemia tissues may be useful for distinguishing ferritins from normal and pathological cases. This work was supported in part by the Russian Foundation for Basic Research (grant # 09-02-00055-a).

P-371
Structural study of the interaction of SopB, a TTSS effector of *Salmonella,* and its chaperone SigE
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Invasion of epithelial cells by *Salmonella enterica* is mediated by bacterial "effector" proteins that are delivered into the host cell by a type III secretion system (TTSS). The collaborative action of these translocated effectors modulates a variety of cellular processes leading to bacterial uptake into mammalian cells. Type III effectors require the presence in the bacterial cytosol of specific TTS chaperones. Effectors are known to interact with their chaperone *via* a Chaperone Binding Domain (CBD) situated at their N-terminus. This work focus on SopB, an effector with phosphoinositide phosphatase activity and particularly its interaction with the specific chaperone SigE by biochemical, biophysical and structural approaches.
We have co-expressed SopB with its specific chaperone SigE and purified the complex, determined the limits of the CBD and purified the $SopB_{CBD}$/SigE complex. The structure of SigE has been solved previously but no crystals could be obtained for structure determination of both complexes. We used SAXS experiment combined with biophysical approach to analyse the interaction between SopB and its chaperone as well as the quaternary structure on the complex that will be described in this presentation.

P-372
A spectroscopic study of GMP kinase secondary structure in interaction with ligands
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Guanylate monophosphate kinase (GMPK) is a cytosolic enzyme involved in nucleotide metabolic pathways. One of

the physiological roles of GMPKs is the reversible phosphoryl group transfer from ATP to GMP (its specific ligand), yielding ADP and GDP. The GMPK from *Haemophilus influenzae* is a small protein, with a number of 208 amino acids in the primary structure.
In order to determine the secondary structure changes of this enzyme, as well as some physical characteristics of its complexes with GMP and ATP ligands, circular dichroism (CD) and ATR - FTIR studies were performed. The enzyme and its ligands were dissolved in Tris - HCl buffer, at pH 7.4 and 25 °C.
From the CD spectra the content of the secondary structure elements of GMPK and GMPK/GMP, GMPK/ATP (with and without Mg^{2+}) were determined. The major secondary structure elements of GMPK from *Haemophilus influenzae* were α-helix ($\sim$ 37 %) and β-sheet ($\sim$ 36 %). ATR - FTIR experiments show that the amide I and amide II bands of the GMPK are typical for a protein with great α-helix content. From the second derivative spectra, the content of the secondary structure elements were estimated. These data were in agreement with those obtained by CD.

P-374
Helical peptides derived from the dimerization helix of the capsid protein of HIV-1bind protein wt
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Assembly of the mature human immunodeficiency virus type 1 capsid involves the oligomerization of the capsid protein, CA. The C-terminal domain of CA, CTD, participates both in the formation of CA hexamers, and in the joining of hexamers through homodimerization. Intact CA and the isolated CTD are able to homodimerize in solution with similar affinity (dissociation constant in the order of 10 μM); CTD homodimerization involves mainly an α-helical region. In this work, we show that peptides derived from the dimerization helix (which keep residues energetically important for dimerization and with higher helical propensities than the wild-type sequence) are able to self-associate with affinities similar to that of the whole CTD.
Moreover, the peptides have a higher helicity than that of the wild-type sequence, although is not as high as the theoretically predicted. Interesting enough, the peptides bind to CTD, but binding in all peptides, but one, does not occur at the dimerization interface of CTD (helix 9). Rather, binding occurs at the last helical region of CTD, even for the wild-type peptide, as shown by HSQC-NMR. As a consequence, all peptides, but one, are unable to inhibit capsid assembly of the whole CA *in vitro*. The peptide whose binding occurs at the CTD dimerization helix has an Val→Arg mutation in position 191, which has been involved in dimer-dimer contacts. These findings suggest that event keeping the energetically important residues to attain CTD dimerization within a more largely populated helical structure is not enough to hamper dimerization of CTD.

P-375
Structure and Function of the Sodium/Proline Transporter PutP studied by EPR Spectroscopy
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PutP is an integral membrane protein located in the cytoplasmic membrane of *Escherichia coli*, being responsible for the coupled transport of Na^+ and proline. It belongs to the family of sodium solute symporters (SSS). Structural data for PutP is not available, but secondary structure predictions together with biochemical and biophysical analyses suggest a 13 transmembrane motif. From a recent homology model based on the X-ray structure of the related Na^+/galactose symporter vSGLT, previously published electron paramagnetic resonance (EPR) studies, and recent crystallographic and EPR studies on the cognate bacterial homolog of a neurotransmitter:Na^+ symporter, LeuT, it has been proposed that helices VIII and IX as well as the interconnecting "loop 9" region determine the accessibility of the periplasmic cavities which bind sodium and proline.
We performed site-directed spin labeling of "loop 9" in combination with EPR spectroscopy to investigate the structural features of this region and possible conformational changes induced by sodium and proline. Analyses of spin label mobility and polarity as well as accessibility to paramagnetic quenchers allow us to refine this region in the present homology model. Furthermore, our data suggest conformational changes in this region upon substrate binding including an overall motion of a helical segment.

P-376
Interactions of Brain Fatty Acid Binding Protein as monitored by site directed spin labeling
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Fatty acid-binding proteins (FABP) are a family of low molecular weight proteins that share structural homology and the ability to bind fatty acids. The common structural feature is a β-barrel of 10 antiparallel β-strands forming a large inner cavity that accommodates nonpolar ligands, capped by a portal region, comprising two short α-helices. B-FABP exhibits high affinity for the docosahexaenoic (DHA) and oleic acid (OA). It is also postulated that B-FABP may interact with nuclear receptors from PPAR family. In the present work, we used molecular biology and spectroscopic

techniques to correlate structure, dynamics and function. Site-directed mutagenesis was used to produce 5 mutants of B-FABP with a nitroxide spin probe (MTSL) selectively attached to residues located at the portal region. ESR spectra of the labeled B-FABP mutants were sensitive to the location of the mutation and were able to monitor interactions in three cases: (1) FABP in the presence of DHA and OA ligands; (2) FABP in the presence of POPC and POPG lipid model membranes, where we evidenced the importance of electrostatic interactions and a possible collisional mechanism for the delivery of FA; (3) FABP in the presence of PPAR nuclear receptor, in which case we were able to detect the complex formation.

P-377

Effect of DNA binding on the self-association of the human recombinase protein Rad51

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HsRad51 is a protein that plays key role in the homologous recombination repair (HRR) of double strand DNA breaks (DSB) in human cells. It has been found that the presence of Rad51 homologues is essential for cell viability in higher eukaryotes. It is supposed that in vivo, the recombination process is initiated by the formation of 3'-ssDNA overhangs which are then recognized by Rad51 with the help of other interacting proteins. Rad51 then forms a helical nucleoprotein filament around ssDNA. In concert with other key players the nucleoprotein filament then binds the dsDNA substrate, recognizes homology and exchanges the DNA strands in an ATP dependent manner. It was shown that in higher eukaryotes HRR requires the presence of the breast cancer associated protein BRCA2. Based on co-crystallization and structure determination of the complex of BRCA2 and the ATP-binding domain of HsRad51 it was suggested that the self-association of Rad51 is regulated by the repeats of BRCA2 intruding into the interface of polymer formation (Pellegrini et al. *Nature*, (2002) 420, 287-293). Indeed, the interface of homo-polymerization may be an important site of the regulation of filament formation.

The present study was based on the reported finding that in the absence of DNA, Rad51 forms ring-like self-aggregates in solution. In the experiments, HsRad51 protein and its mutants were produced by bacterial expression and purified in our laboratory. The ring-like associates were characterized by dynamic light scattering and AFM. Binding to ssDNA and dsDNA was verified by fluorescence spectroscopy and single molecule techniques. Fluorescence label studies combined with high pressure technique were used to determine the dissociation constant of the interface (Schay et al., *JBC* (2006) 281, 25972-25983) and its dependence on the solution components. The fluorescence high pressure technique was applied to study the role of the protein interface interaction in the nucleoprotein formation.

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P-378

New insight in the assembly and function of human small heat shock proteins (sHSP)

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sHSP are ubiquitous proteins involved in cellular resistance to various stress (oxidative, heat, osmotic…). They are able to prevent aggregation of non-native proteins through the ability of forming large soluble complexes and preventing their non-specific and insoluble aggregation. In consequence to this molecular chaperone function, they can regulate many processes (resistance to chemotherapy, modulation of the cellular adhesion and invasion, inflammatory response in skin), and the modulation of their expression has been found to be a molecular marker in cancers, spermatogenesis, or cartilage degeneration. Furthermore, they are involved in several pathologies: myopathies, neuropathies, cancers, cataracts.

Among the 10 human members (HspB1-10), this study focused on HspB1 (Hsp27 involved in some cancers), HspB4 (lens specific), HspB5 (lens, muscle, heart, lung) and HspB5-R120G (responsible for a desmin-related myopathy and a cataract). As sHSP form large, soluble (but polydisperse in mammals) hetero-oligomers, molecular biology, biochemistry, biophysics and bioinformatics were successfully combined to compare the functional/dysfunctional assemblies in order to understand the critical parameters between sHSP members depending upon their tissue and cellular localization.

P-379

Molecular damage caused by radiation exposure

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Ionizing radiation is a type of radiation that contains enough energy to displace electrons and break chemical bonds. This can promote the removal of at least one electron from an atom or molecule, creating radical species, namely, Reactive Oxygen Species (ROS) [1, 2]. These are often associated with damages at cellular level, such as, DNA mutations, cell cycle modifications and, in animal cells, cancer. To overcome this problem, organisms developed different protection/repair mechanisms that enable them to survive to these threats. DNA glycosylases are enzymes that are part of base excision repair (BER) system, mainly responsible for DNA repair. They can recognize a DNA lesion and, in some cases, are able to remove the mutated base. Here we propose to study one of those enzymes, endonuclease III, which contains a [4Fe-4S] cluster [3, 4]. Samples were

exposed to different doses of UV-C radiation and the effects were studied by electrophoretic and spectroscopic methods.

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P-380

Cholesterol affects Na,K-ATPase thermostability and catalytic activity

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Na,K-ATPase is an integral protein present in the plasma membrane of animal cells, and consists of two main subunits: the α and β. Cholesterol is an essential constituent of the animal membrane cells. In order to study the interaction between Na,K-ATPase and cholesterol, we have used the DSC technique, and a proteoliposome system composed by the enzyme and DPPC:DPPE, with different percentage in mol of cholesterol. The heat capacity of purified Na,K-ATPase profile exhibits three transitions with 31, 189 and 60 kcal/mol at 55, 62 and 69°C. Multiple components in the unfolding transition could be attributed either to different steps in the pathway or to independent unfolding of different domains. This denaturation of Na,K-ATPase is an irreversible process. For the proteoliposome, we also observed three peaks, with 180, 217 and 41 kcal/mol and 54, 64 and 72°C. This increase in ΔH indicates that the lipids stabilize the protein. When cholesterol was used (from 10 to 40 mol %), the first transition was shifted to a lower temperature value around 35°C. These results confirm that cholesterol has an influence on the packing and fluidity of lipid bilayer and changes in lipid microenvironment alter the thermostability as well as the activity of Na,K-ATPase. Financial support: FAPESP.

P-381

Apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia alter the protein's thermodynamic properties

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Apolipoprotein E is a major lipid carrier protein in humans, plays important roles in lipid homeostasis and protection from atherosclerosis. ApoE is characterized by structural plasticity and thermodynamic instability and can undergo significant structural rearrangements as part of its biological function. Mutations in the LDL binding region 136-150 of apoE have been linked with lipoprotein disorders such as Type III Hyperlipoproteinemia in humans and experimental animals. In this study we examined whether three such mutations, namely R136S, R145C and K146E affect the thermodynamic stability and conformation of apoE3. Circular dichroism spectroscopy revealed that the mutations do not alter the secondary structure of the protein. However, all three mutants have altered thermodynamic stability compared to wild-type as evidenced by thermal and chemical denaturation experiments. Furthermore, all three variants are able to clarify DMPC emulsions, but with subtle changes in kinetics that suggest differences in equilibrium distribution of conformations. Overall, our findings suggest that single amino acid changes in the functionally important region 136-150 of apoE3 can affect the molecule's stability and structural lability in solution. We propose that these thermodynamic alterations be taken into account when evaluating the pathogenic potential of apoE3 variants.

O-382

Structure-function relationships in different MFE-2 proteins by various biophysical techniques

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We have undertaken to study the structure and function of peroxisomal multifunctional enzyme type 2 (MFE-2) from different organisms. MFE-2 is a key enzyme in long- and branched-chain fatty acids breakdown in peroxisomes. It contains two enzymes in the same polypeptide and also consists of differing amount of domains depending on the species. Crystal structure and enzyme kinetics of *Drosophila melanogaster* MFE-2 has revealed the domain assembly and raised a question about existence of a substrate channeling mechanism. Small-angle x-ray scattering studies have further resolved the assembly of domains in the human MFE-2. Mutations in the MFE-2-coding gene in humans may cause D-bifunctional protein deficiency - a metabolic disease characterized by accumulation of fatty acyl-CoA intermediates due to inactive or residually active MFE-2 protein. We have also studied the structure, stability and dynamics of such mutant proteins both experimentally and *in silico*. The latest results on all these studies will be presented.

P-383
Self-organisation of FtsZ protein filaments on a substrate
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FtsZ is a protein that plays a key role in bacterial division, forming a protein ring directly related to the constriction of the membrane. This process has been observed to occur without the help of molecular motors. Nonetheless, the details of the self-assembly and subsequent force generation of the septal ring are still obscure.
AFM observations allows the study of the behaviour of FtsZ solutions on a substrate with unprecedented resolution, permitting the identification of individual protein filaments. The different resulting structures can be compared to Monte Carlo models for a 2D lattice accounting for the essential interactions between monomers. These include a strong longitudinal bond that allows a limited flexibility (i.e., curvature of the filaments) and a weaker lateral interaction.
The work we present follows this approach, focussing on the latest experiments with FtsZ mutants. By using these mutants, it is possible to choose the specific region of the monomer that will anchor to the substrate, thus generating new structures that provide an insight into monomer-monomer interactions. In this way, we explore the anisotropy of the lateral bond in FtsZ, a factor that has not been taken into account before but may prove to be of importance in FstZ behaviour *in vivo*.

P-384
Modeling protein structures and their complexes with limited experimental data
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Conventional methods for protein structure determination require collecting huge amounts of high-quality experimental data. In many cases the data (possibly fragmentary and/or ambiguous) on itself cannot discriminate between alternative conformations and a unique structure cannot be determined. Small Angle Xray Scattering is an example of such a “weak” experiment. The spectrum encodes only several independent degrees of freedom that provide a global description of a molecular geometry in a very synthetic way. In this contribution we utilized both local information obtained from nmR measurements and global description of a macromolecule as given by SAXS profile combined with a knowledge-based bimolecular force field to determine tertiary and quaternary structure of model protein systems. SAXS curve as well as various kinds of local nmR data such as isotropic Chemical Shifts and their tensors, J-couplings, RDC, backbone NOE and REDOR from nmR in solid phase are parsed with the ‘‘experimental’’ module of BioShell toolkit and utilized by Rosetta modeling suite to generate plausible conformations. Obtained results show the new protocol is capable to deliver very accurate models.

P-385
NOEnet—use of NOE networks for nmR resonance assignment of proteins with known 3D structure
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Structural genomics programs today yield an increasing number of protein structures, obtained by X-Ray diffraction, whose functions remain to be elucidated. nmR plays here a crucial role through its ability to readily identify binding sites in their complexes or to map dynamic features on the structure. An important nmR limiting step is the often fastidious assignment of the nmR spectra. For proteins whose 3D structures are already known, the matching of experimental and back-calculated data allows a straightforward assignment of the nmR spectra.
We developed NOE*net*, a structure-based assignment approach. It is based on a complete search algorithm, robust against assignment errors, even for sparse input data. It allows functional studies, like modeling of protein-complexes or protein dynamics studies for proteins as large as 28 kD.
Almost any type of additional restraints can be used as filters to speed up the procedure or restrict the assignment ensemble. NOE*net* being mainly based on nmR data (NOEs) orthogonal to those used in triple resonance experiments (J-couplings), its combination even with a low number of ambiguous J-coupling based sequential connectivities yields a high precision assignment ensemble.

P-386
Temperature adaptation requires specific changes in the conformational flexibility pattern of IPMDH
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We observed, that *T. thermophilus* isopropylmalate dehydrogenase (IPMDH) has higher rigidity and lower enzyme activity at room temperature than its mesophilic counterpart *(E. coli),* while the enzymes have nearly identical flexibilities under their respective physiological working conditions, suggesting that evolutionary adaptation tends to maintain optimum activity by adjusting a “corresponding state” regarding conformational flexibility. In order to reveal the nature of the conformational flexibility change related to enzymatic activity, we designed a series of mutations involving non conserved prolines specific to thermophilic IPMDH. Proline to glycine mutations substantially increased conformational flexibility and decreased conformational stability. The mutant enzyme variants did not show enhanced catalytic activity, but the non Arrhenius temperature dependence of enzyme activity of the wild type was abolished. This phenomenon underlines the fact that the delicate balance between flexibility, stability and activity which is required for the environmental adaptation of enzymes can be easily disrupted with mutations even distant from the active site, providing further evidence that optimization of proper functional motions are also a selective force in the evolution of enzymes.

P-388

Structures of *Trypanosoma brucei* dUTPase provide insight into the reaction mechanism

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The kinetoplastids *Trypanosoma brucei*, *T. cruzi* and *Leishmania major* are responsible for causing great morbidity and mortality in developing countries. The all α-helical dimeric dUTPases from these organisms represent promising drug targets due to their essential nature and markedly different structural and biochemical properties compared to the trimeric human enzyme. To aid in the development of dUTPase inhibitors we have been structurally characterizing the enzymes from these species. Here we present the structure of the *T. brucei* enzyme in open and closed conformations, completing the view of the enzymes from the kinetoplastids. Furthermore, we sought to probe the reaction mechanism for this family of enzymes as a mechanism has been proposed based on previous structural work but has not received any further verification. The proposed scheme is similar to that of the trimeric enzyme but differs in detail. Using tryptophan fluorescence quenching in the presence of the transition state mimic AlF_3 we have been able to identify which species is the likely transition state in the reaction. The crystal structure of *T. brucei* in complex with this transition state analogue confirms the nature of the nucleophilic attack clearly showing how it differs from trimeric enzymes.

O-389

The structure of factor H-C3d complex explains regulation of immune complement alternative pathway

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The alternative pathway of complement is important in innate immunity, attacking not only microbes but all unprotected biological surfaces through powerful amplification. It is unresolved how host and nonhost surfaces are distinguished at the molecular level, but key components are domains 19-20 of the complement regulator factor H (FH), which interact with host (i.e., nonactivator surface glycosaminoglycans or sialic acids) and the C3d part of C3b. Our structure of the FH19-20:C3d complex at 2.3 Å resolution shows that FH19-20 has two distinct binding sites, FH19 and FH20, for C3b. We show simultaneous binding of FH19 to C3b and FH20 to nonactivator surface glycosaminoglycans, and we show that both of these interactions are necessary for full binding of FH to C3b on nonactivator surfaces (i.e., for target discrimination). We also show that C3d could replace glycosaminoglycan binding to FH20, thus providing a feedback control for preventing excess C3b deposition and complement amplification. This explains the molecular basis of atypical hemolytic uremic syndrome, where mutations on the binding interfaces between FH19-20 and C3d or between FH20 and glycosaminoglycans lead to complement attack against host surfaces.

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Improved secondary structure determination of proteins with SRCD: distinguishing parallel and antiparallel β-structure

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Circular Dichroism (CD) spectroscopy is a widely used technique for studying the secondary structure (SS) of proteins. Numerous algorithms have been developed for the estimation of the SS composition from the CD spectra. Although, these methods give more or less accurate estimation for proteins rich in α-helical structure, they often fail to provide acceptable results on mixed or β-rich proteins. The problem arises from the diversity of β-structures, which is thought to be an intrinsic limitation of the technique. The worst predictions are provided for proteins of unusual β-structures and for amyloid fibrils. Our aim was to develop a new algorithm for the more accurate estimation of SS contents for a broader range of protein folds with special interest to amyloid fibrils. Using synchrotron radiation CD (SRCD), we were able to collect high quality spectra of amyloid fibrils with good S/N ratios down to 175nm. The novel reference dataset with spectra that significantly differ from present reference sets, extends the information content for SS determination. Our algorithm takes into account the diverse twist of the β-sheets that has a great influence on the spectral features. For the first time, we can reliably distinguish parallel and antiparallel β-structure using CD spectroscopy.

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Monitoring the assembly of the membrane protein insertase

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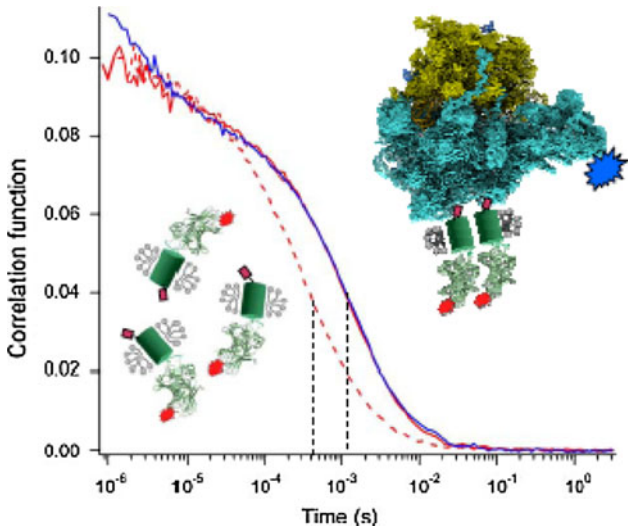
Molecular forces that govern membrane protein integration and folding remain a major question in current molecular biology and biophysics. Each nascent polypeptide chain should acquire its unique three-dimensional folded state within a complex environment formed by the anisotropic lipid membrane and the membrane-water interface. SecYEG translocase and members of a recently described YidC/Oxa1/Alb3 chaperone family are recognized as primary players in the membrane protein genesis. These proteins, so called insertases, serve as membrane-embedded molecular pores where the newly synthesized protein is loaded prior its release into the bilayer. Here we apply fluorescence correlation spectroscopy to monitor the assembly

of the insertase:ribosome:nascent polypeptide chain complexes in solution and reconstituted into nanodiscs and model membranes. Results provide insights on molecular mechanisms and dynamics of the insertase functioning.



O-392

Conformational changes during GTPase activity induced self-assembly of human guanylate binding protein 1 revealed by EPR spectroscopy

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Human guanylate binding protein 1 (hGBP1) belongs to the Dynamin Superfamily of large GTPases. hGBP1 is an elongated 67 kDa protein comprising three distinct domains, a large GTPase (LG) domain, the middle domain - a five-helix bundle, and the GTPase effector domain (GED) consisting of two helices. The latter subdomain flanks the whole protein and has contacts with the LG domain.

During nucleotide hydrolysis the protein forms oligomers which lead to a self stimulation of its GTPase reaction. For this reason hGBP1 can be categorized as G protein activated by nucleotide-dependent dimerization (GAD). Up to date, no structural information about about the overall conformation of the full-length protein in the predicted dimeric or tetrameric state could be obtained.

To the structural properties of hGBP1 upon oligomerization, we applied site-directed spin labeling (SDSL) in combination with electron paramagnetic resonance (EPR) spectroscopy. We used continuous wave and pulse EPR methods, namely Double Electron Electron Resonance (DEER) spectroscopy, to determine distance distributions between spin label side chains. We used these distance constraints to model the arrangement of hGBP1 in the dimeric state and conformational changes, especially of the GED, taking place upon dimerization.

O-393

Structural insights into the control of type three secretion

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Correct assembly and regulation of multi-component molecular machines is essential for many biological activities. The Type III Secretion System (T3SS) is a complex molecular machine that is a key virulence determinant for important Gram-negative pathogens including *Shigella, Yersinia* and *Salmonella* species [1-2]. The T3SS consists of multiple copies of ~25 different proteins (totalling ~7MDa), spans both bacterial membranes and drives insertion of a contiguous pore into the host-cell membrane. Virulence factors are secreted via this apparatus directly into the host cell.

In all T3SS various levels of regulation occur with switching between secretion off and on states overlaid on control of which substrates are secreted. Genes involved in a variety of these switches have been identified but the molecular mechanisms underlying these functions is poorly understood. We are studying the T3SS of *Shigella flexneri*, the causative agent of dysentery and will present the structure of the so-called "gatekeeper protein" MxiA.

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P-394

Circular dichroism and the secondary structure of the ROF2 protein from *Arabidopsis thaliana*

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The protein ROF2 from the plant *Arabidopsis Thaliana* acts as a heat stress modulator, being involved in the long-term acquired thermotolerance of the plant. Here we investigate the relationship between the biological function and the structure of ROF2 by circular dichroism (CD) spectroscopy in the far and near UV domains. By analysing our CD spectrua with the CDPro software developed by Sreerama et al. (2000), we have obtained the percentages of α-helices, β-sheets, and turns in the secondary structure of ROF2. The near UV results suggest that ROF2 proteins can associate, forming super-secondary structures. CD experiments performed at temperatures between 5 °C and 97 °C indicate that the thermal denaturation of ROF2 caused by a raise in

temperature up to 55 °C is followed by a partial recovery of the secondary structure as the temperature is raised further. This reversibility of the thermal denaturation of ROF2 might play an important role in the thermotolerance of *Arabidopsis Thaliana*.

P-395
Investigation of the bovine DGAT1 enzyme binding sites specificity

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The diacylglycerolacyltransferase1 (DGAT1) is an integral protein from the reticulum endoplasmic membrane that plays an essential role in triacylglyceride synthesis. In cattle, this enzyme is associated to the fat content regulation on milk and meat. In this study, synthetic peptides corresponding to both DGAT1 binding sites (Sit1 and Sit2) were designed, purified and employed to investigate the enzyme interaction with substrates and membrane models. Different binding specificities in the interaction with phospholipid vesicles and micelles were noted. Sit1 showed to bind more strongly in nonpolar membrane models, while Sit2 was electrostatically attracted to negative phospholipid surfaces. The binding of both peptides was followed by significant conformational changes (like unordered to helix transition) in circular dichroism spectra and a 20nm blue shift in fluorescence emission. The binding of Sit1 and Sit2 peptides to negative liposome gave dissociation constants (K_D) of 170 and 0.44μM, respectively, and a leakage action 24-fold higher to Sit2. The difference in specificity is related to the features of the putative substrates (acyl-CoAs and diacylglycerol) and can be attributed to the distinct role of each DGAT1 binding site during lipid synthesis. Supported by FAPESP.

P-396
Molecular dynamics simulation of short polymers of the cell division protein FtsZ suggests that GTPase activity is restricted to filament ends

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FtsZ, the bacterial homologue of tubulin, assembles into polymers in the bacterial division ring. The interfaces between monomers include a GTP molecule, although the relationship between polymerization and GTPase activity is still controversial. A set of short FtsZ polymers were modelled and the formation of active GTPase structures was monitored using molecular dynamics. Only the interfaces nearest the polymer ends exhibited geometry adequate for GTP hydrolysis. Conversion of a mid-polymer interface to a close-to-end interface resulted in its spontaneous rearrangement from an inactive to an active conformation.

P-397
Reversible and irreversible photoactivation in mIrisFP variants

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Fluorescent proteins (FPs) have become extremely valuable tools in the life sciences. Due to the latest advances in the light microscopy, there is a steady need for FPs with improved spectral properties. mIrisFP is a monomeric FP that can be switched reversibly between a bright green fluorescent and a dark state by illumination with light of specific wavelengths [1]. Structurally, this photo-switching is based on a cis-trans isomerization of the chromophore. Upon illumination with violet light, mIrisFP can be irreversibly photoconverted from the green-emitting to a red-emitting form. The red form can again be switched reversibly between a fluorescent and a dark state. To elucidate the mechanistic details of the photoinduced reactions, we have generated mIrisGFP1. This variant can still undergo reversible photoswitching, but lacks the ability to photoconvert to the red state so that the photoinduced transitions of the green form can be studied without 'artifacts' due to green-to-red photoconversion. Using UV/visible spectroscopy, we have characterized the on- and off- switching processes in great detail. Several light-activated reaction pathways have been identified. They are highly intertwined so that the net effect achieved with light of a particular wavelength depends on the relative probabilities to photoinduce the various processes.

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P-398
Biophysical study of the complex between the 14-3-3 protein and phosducin

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Phosducin (Pd) is a $G_t\beta\gamma$-binding protein that is highly expressed in photoreceptors. Pd is phosphorylated in dark-adapted retina and is dephosphorylated in response to light. Dephosphorylated Pd binds G_t protein $\beta\gamma$-heterodimer with high affinity and inhibits its interaction with $G_t\alpha$ or other effectors, whereas phosphorylated Pd does not. Therefore Pd down-regulates the light response in photoreceptors. Phosphorylation of Pd at S54 and S73 leads to the binding of the 14-3-3 protein. The 14-3-3

proteins function as scaffolds modulating the activity of their binding partners and their role in Pd regulation is still unclear. The 14-3-3 protein binding may serve to sequester phosphorylated Pd from $G_t\beta\gamma$ or to decrease the rate of Pd dephosphorylation and degradation. We performed several biophysical studies of the 14-3-3:Pd complex. Analytical ultracentrifugation was used to determine the complex stoichiometry and dissociation constant. Conformational changes of Pd induced both by the phosphorylation itself and by 14-3-3 binding were studied using the time-resolved fluorescence spectroscopy techniques. This work was funded by Grant P305/11/0708 of the Czech Science Foundation and by Research Project MSM0021620857 of the Ministry of Education, Youth, and Sports of the Czech Republic.

P-399

BMH-dependent activation of yeast enzyme neutral trehalase

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Trehalase (EC 3.2.1.28) is a glycoside hydrolase enzyme that catalyzes the conversion of α, α-trehalose to two molecules of glucose. Yeast BMH1 and BMH2 proteins (yeast 14-3-3 isoforms) form a complex with neutral trehalase (NTH1) after its phosphorylation by PKA. We showed that PKA phosphorylates NTH1 *in vitro* on four Serine residues: 20, 21, 60 and 83. To find out which site or sites are essential for the BMH binding we produced NTH1 WT (both phosphorylated and non-phosphorylated), four NTH1 mutants containing single phosphorylation site, one double phosphorylated NTH1 mutant (at Ser20 and 21) and a mutant containing none of these studied phosphorylation sites as well. The interaction between BMH1 and BMH2 protein with enzyme NTH1 was monitored using native electrophoresis and sedimentation velocity measurements. The sedimentation equilibrium analysis was used to define the stoichiometry of NTH1/BMH complexes. Finally, we used enzyme kinetic measurements to monitor the BMH-dependent activation of NTH1.
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P-400

Mössbauer spectroscopy of various oxyhemoglobins in relation to their structure and functions

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Mössbauer spectroscopy with a high velocity resolution was used for comparative study of various oxyhemoglobins for analysis of the heme iron electronic structure and protein structure-function relationship. Samples of pig, rabbit and normal human oxyhemoglobins and oxyhemoglobins from patients with chronic myeloleukemia and multiple myeloma were measured using Mössbauer spectrometric system with a high velocity resolution at 90 K. Mössbauer spectra were fitted using two models: one quadrupole doublet (model of equivalent iron electronic structure in α– and β–subunits of hemoglobins) and superposition of two quadrupole doublets (model of non-equivalent iron electronic structure in α– and β–subunits of hemoglobins). In both models small variations of Mössbauer hyperfine parameters (quadrupole splitting and isomer shift) were observed for normal human, rabbit and pig oxyhemoglobins and related to different heme iron stereochemistry and oxygen affinity. Small variations of Mössbauer hyperfine parameters for oxyhemoglobins from patients were related to possible variations in the heme iron stereochemistry and function.
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P-401

Structure-function relationships in spider silk

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The different types of silk produced by orb-weaving spiders display various mechanical properties to fulfill diverse functions. For example, the dragline silk produced by the major ampulate glands exhibits high toughness that comes from a good trade-off between stiffness and extensibility. On the other hand, flagelliform silk of the capture spirals of the web is highly elastic due to the presence of proline and glycine residues. These properties are completely dictated by the structural organization of the fiber (crystallinity, degree of molecular orientation, secondary structure, microstructure), which in turn results from the protein primary structure and the mechanism of spinning. Although the spinning process of dragline silk begins to be understood, the molecular events occurring in the secretory glands and leading to the formation of other silk fibers are unknown, mainly due to a lack of information regarding their initial and final structures. Taking advantage of the efficiency of Raman spectromicroscopy to investigate micrometer-sized biological samples, we have determined the conformation of proteins in the complete set of glands of the orb-weaving spider *Nephila clavipes* as well as in the fibers that are spun from these glands.

P-402

Structural study of the 14-3-3/RGS3 protein complex

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In this study we were particularly interested in 14-3-3 ζ / RGS3 protein complex. The crystal structure of RGS

domain of RGS3 at 2.3Å resolution was solved. The stoichiometry of 14-3-3 ζ /RGS3 protein complex was elucidated using the analytical ultracentrifugation. To map the interaction between 14-3-3ζ and RGS3 protein we performed a wide range of biophysical measurements: H/D exchange and cross link experiments coupled to mass spectrometry, FRET (Förster resonance energy transfer) time-resolved fluorescence experiments, time-resolved tryptophan fluorescence spectroscopy and SAXS (small angle X-ray scattering) measurements. Based on all these results we build 3D model of 14-3-3 ζ /RGS3 protein complex. Our model revealed new details on architecture of complex formed by 14-3-3 proteins. To date all known structure of 14-3-3 proteins complexes suggests that the ligand is docked in the central channel of 14-3-3 protein. Our results indicate that the RGS domain of RGS3 protein is located outside the central channel of 14-3-3ζ protein interacting with less-conserved residues of 14-3-3ζ.
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P-403
Solution structure and self assembly properties of soluble RAGE
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The Receptor for Advanced Glycation End-products (RAGE) is a multiligand cell surface receptor involved in various human diseases. The major alternative splice product of RAGE comprises its extracellular region that occurs as a soluble protein (sRAGE). Although the structures of sRAGE domains were available, their assembly into the functional full length protein remained unknown. Here we employed synchrotron small-angle X-ray scattering to characterize the solution structure of human sRAGE. The protein revealed concentration-dependent oligomerization behaviour, which was also mediated by the presence of Ca^{2+} ions. Rigid body models of monomeric and dimeric sRAGE were obtained from the scattering data recorded at different solvent conditions. The monomer displays a J-like shape while the dimer is formed through the association of the two N-terminal domains and has an elongated structure. The results provided insight into the assembly of i) the heterodimer sRAGE:RAGE, which is responsible for blockage of the receptor signalling, and ii) RAGE homodimer, which is necessary for signal transduction, paving the way for the design of therapeutical strategies for a large number of different pathologies.

O-406
Three-dimensional structure of ClpB, a chaperone that rescues aggregated proteins
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ClpB is a hexameric AAA+ ATPase that extracts unfolded polypeptides from aggregates by threading them through its central pore. The contribution of coiled-coil M domains is fundamental for the functional mechanism of this chaperone, and its location within the protein structure in previous structural models is contradictory. We present cryo-electron microscopy structural analysis of ClpB from *E. coli* in several nucleotide states. The study reveals a novel architecture for ClpB and shows that M domains form an internal scaffold located in the central chamber of ClpB hexamers. This inner structure transmits local signals due to ATP binding and hydrolysis by AAA+ domains. Surprisingly, coiled-coil M domains are seen to bend significantly around a hinge region that separates two structural motifs. Our results present a new framework to understand ClpB-mediated protein disaggregation.

P-407
***Streptomyces clavuligerus* isoenzymes involved in clavulanic acid biosynthesis: a structural approach**
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Clavulanic acid (CA) is a potent β-lactamase inhibitor produced by *Streptomyces clavuligerus*. N^2-(2-carboxyethyl) arginine synthase (CEAS) and proclavaminate amidino hydrolase (PAH) catalyze the initial steps in the biosynthesis of CA. Recently *ceas*1and *pah*1genes (paralogous of *ceas* and *pah*) were related to the CA biosynthesis but their products have not been studied yet. Here we present the initial structural analysis of CEAS1 and PAH1 using biophysical techniques. *Pah1* and *ceas1* genes were isolated from the genomic DNA of *S. clavuligerus* and overexpressed in *E. coli*. The recombinant proteins were purified by affinity chromatography and analyzed by size exclusion chromatography, non-denaturing PAGE, dynamic light scattering, far-UV Circular Dichroism (CD) and fluorescence spectroscopy. Our results showed that PAH1 and CEAS1 were obtained as hexamer and dimer respectively. Both proteins showed an α/β folding, being stable up to 35°C. Above this temperature protein unfolding was observed but *the complete unfolding* was *not observed,* even at 100°C. Moreover CEAS1 and PAH1 showed to be stable over a wide pH range (pH 5.5 – 9.5). We are currently working on improving CEAS1 crystals which are a promising step towards the elucidation of the CEAS1 structure. Supported by FAPESP.

O-408

Structural and biochemical studies show divergent allosteric mechanisms in glycoytic enzymes

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We have solved a series of eukaryote pyruvate kinase X-ray structures from the parasite Leishmania mexicana (*Lm*PK) and the human M1 (*hs*M1PK) and M2 (*hs*M2PK) isoforms. Despite identical active sites and similar overall tetramer architecture, the details of allosteric activation are surprisingly different.

The parasite enzymes use the effector molecule fructose 2,6 bisphosphate (F26BP).A series of four *Lm*PYK crystal structures solved in all possible ligated states (+/- F26BP and substrate +/- F26BP) shows that enzyme activation is the result of a conformational change, whereby a concerted rocking motion primes the active sites. This activated state is locked by binding the F26BP effector; this 'rock-and-lock' mechanisms explains activating role of effector which binds over 40 Å away from the active site.

In mammals the M2 isoform is found in rapidly proliferating (e.g. embryonic and cancer) cells and is allosterically regulated by F16BP. The M1 isoform is an alternative spliced variant and is constitutively active and differs in only 22 amino acids. A direct comparison of the first X-ray crystal structure of *hs*M1PK with *hs*M2PK (crystallised under identical conditions) shows how the 22 different amino acids affect the tetramer interface. Together with light scattering and gel filtration data we show that allosteric regulation in M2 PK is best explained in terms of a monomer-tetramer equilibrium.

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DISCO: dichroism, imaging & spectrometry for chemistry and biology

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DISCO at SOLEIL Synchrotron (France) is a VUV to visible beamline and operational since Sept. 2009. Three endstationsserve the biology and chemistry communities resolving the common scientific topic of Bio- macromolecular structure interactions.

- Synchrotron Radiation Circular Dichroism
- Mass spectrometry following VUV photoionisation
- Fluorescence imaging with lifetime and spectral measurements

Here we will present the SRCD experiment. High photon flux of 10^{10}photons / sec, improved detector performances as well as user-orientated software developments have proven to be the garants for successful data collections,which considerably increased the information content obtained. The exploration into the charge transfer region of the peptide bonds is adding specifically new insights. Low sample volumes of as little as 2μl per spectra as well as convenient sample chamber handling allow for economic and efficient data collections. **Typical spectra acquisition from 280 to 170nm, last for 9 min for three scans with a 1nm step size.** Prior to high resolution based techniques, SRCD spectrawill answer questions about folding states of macromolecules including DNA, RNA and sugar macromolecules as well as their complexes with proteins and specially membrane proteins.

O-410

Structure of an intercellular channel formed during sporulation in *Bacillus subtilis*

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Sporulation in *Bacillus subtilis* begins with an asymmetric cell division producing a smaller cell called the forespore, which initially lies side-by-side with the larger mother cell. In a phagocytosis-like event, the mother cell engulfs the forespore so that the latter is internalised as a cell-within-a-cell. Engulfment involves the migration of the mother cell membrane around the forespore until the leading edges of this engulfing membrane meet and fuse. This releases the forespore, now surrounded by a double membrane, into the mother cell cytoplasm.

Membrane migration during engulfment is facilitated by the interacting proteins SpoIIQ and SpoIIIAH that are membrane-associated and expressed in the forespore and the mother cell respectively [1]. They interact in the intercellular space and function initially as a molecular zipper and later they participate in a more elaborate complex in which SpoIIQ and SpoIIIAH are integral components of an intercellular channel. This channel is a topic of much current interest, having initially been proposed as a conduit for the passage from the mother cell to the forespore of a specific, but putative, regulator of the RNA polymerase sigma factor, σ^G [2] and later as a gap junction-like feeding tube [3] through which the mother cell supplies molecules for the biosynthetic needs of the forespore. Here we present data on the structure and interactions of SpoIIQ and SpoIIIAH gleaned from biophysical methods and protein crystallography. These data lead to a plausible model for the intercellular channel.

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2. Meisner, J., Wang, X., Serrano, M., Henriques, A. O. & Moran, C. P. J. (2008). A channel connecting the mother cell and forespore during bacterial endospore formation. *Proc. Natl. Acad. Sci.* **105**, 15100-15105.
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P-411
Kinetic studies of vimentin early assembly and filament elongation
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In vitro assembly of intermediate filament (IF) proteins is a rapid three-step process. First, tetrameric subunits associate laterally into unit length filaments (ULFs). In the second step filaments elongate by longitudinal annealing via end-to-end fusion of their ends. Third, these filaments compact radially into smooth IFs. Here, we analyzed the first two steps. The rapid lateral assembly kinetics of human recombinant vimentin were studied in a stopped-flow device with light scattering detection. By comparing wild type vimentin and a mutant that remains in ULF state, the contributions of the lateral and longitudinal assembly to the light scattering signal could be separated. Longitudinal assembly of vimentin IFs was examined by analyzing the time-dependent length distribution by total internal reflection fluorescence microscopy (TIRFM). These profiles are used to develop robust models that describe the time-dependent filament length distribution of IFs. In order to visualize the mechanisms of late elongation directly, we mixed short filaments containing either Alexa 488- or Alexa 647-labeled vimentin for further assembly. The striped structure of the filaments thus obtained unambiguously showed that IFs elongate through direct end-to-end annealing of shorter filaments.

Live cell imaging

P-414
Dynamics and organization of alpha3-containing glycine receptors using single molecule detection
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The glycine receptor (GlyR) is a chloride permeable ligand gated ion channel and that can mediate synaptic inhibition. Due to a possible involvement in the pathophysiology of temporal lobe epilepsy, the different properties of GlyRs containing alpha3L and K subunit isoforms are currently investigated. Previous characterizations of homomeric receptors consisting of these isoforms have shown a difference in their electrophysiological properties and their membrane distribution as observed by diffraction-limited fluorescence microscopy. We studied these isoforms, when separately expressed in transfected HEK 293T cells, by using single molecule tracking (SMT) in living cells and direct stochastic optical reconstruction microscopy (dSTORM) on fixated cells. For both techniques the GlyRs are stained using a primary antibody directly labeled with Alexa Fluor dyes. The dSTORM experiments support the observation that alpha3L GlyR are clustered, while the alpha3K GlyRs are more uniformly spread. The analysis of the short range diffusion coefficients obtained by SMT reveals the presence of heterogeneous motion for both isoforms. The K-isoform has a higher fraction of fast diffusion. In contrast, the L-isoform is more associated with slow diffusion and appears to undergo hindered diffusion.

P-415
Use of fluorescence microspectroscopy to study nanoparticles delivery into living cancer cells
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Since nanoparticles are suitable for tumor therapy due to their passive targeting to cancer cells by enhanced permeability and retention effect [1], it is important to understand mechanisms of their delivery into the living cancer cells. In this respect we have developed a modular spectral imaging system based on a white light spinning disk confocal fluorescence microscope and a narrow tunable emission filter. Firstly, interaction of polymer nanoparticles and cells labeled with spectrally overlapping probes was examined. The use of fluorescence microspectroscopy (FMS) allowed co-localization, which showed that the size of polymer nanoparticles strongly influences their transfer across the cell plasma membrane. Next, the delivery of liposomes (composed of cancerostatic alkylphospholipid (OPP) and cholesterol) labeled with environment-sensitive fluorescent probe was monitored. We were able to detect a very small shift in emission spectra of cholesterol-poor OPP liposomes inside and outside the cells, which would not be possible without the use of FMS. This shift implies that the delivery of these liposomes into cancer cells is based on fusion with the cell membrane [2].

[1] V. Torchilin, Adv. Drug Deliv. Rev. 63, 131-135 (2011)
[2] Z. Arsov et al., submitted.

O-416
Engineering the excited state of fluorophores for high resolution imaging of bio- and soft-matter
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High-resolution optical imaging techniques make now accessible the detection of nanofeatures in bio-and soft-

matter by non-ionizing visible radiation. However, high-resolution imaging is critically dependent by the fluorescent probes used for reporting on the nano-environment. On account of our long-standing interest in the development of fluorescent probes, we set out to design and engineer new fluorescent systems for nanoscale imaging and sensing of biological specimens and soft-matter. These fluorophores report on fast subtle changes of their nanoscale environment at excited state and are meant to fulfill these requirements: a) optical responses (intensity, wavelength-shift, lifetime, anisotropy) predictably related to the environmental polarity, viscosity, macromolecular structure, b) high brightness allowing for single-molecule detection, c) easily conjugable to biomolecules or macromolecules of interest. Notably, we aim at conjugating these properties with the capability of nanoscopy imaging based on Stimulated Emission Depletion or Stochastic Reconstruction Optical Microscopy. In this lecture the main features and applications of the engineered probes will be reviewed and future developments in this exciting field will be discussed.

P-417

Novel 3D single particle methods reveal new details in the foamy virus entry and fusion processes

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Foamy virus (FV) is an atypical retrovirus which shares similarities with HIV and hepatitis B viruses. Despite numerous biochemical studies, its entry pathway remains unclear, namely membrane fusion or endocytosis. To tackle this issue, dual color fluorescent viruses were engineered with a GFP labeled capsid and a mCherry labeled envelope. Using high resolution 3D imaging and 3D single virus tracing, we followed the entry of the fluorescent viruses in living cells with a precision of 30nm in the plane and 40 nm along the optical axis. To distinguish between the two possible pathways, we developed a novel colocalization analysis method for determining the moment along every single trace where the colors separate, i.e. the fusion event. The combination of this dynamical colocalization information with the instantaneous velocity of the particle and its position within the reconstructed 3D cell shape allows us to determine whether the separation of capsid and envelope happens at the cell membrane or from endosomes. We then compared two types of FV and demonstrated, consistently with previous pH-dependency studies, that the prototype FV can enter the cell by endocytosis and membrane fusion, whereas the simian FV was only observed to fuse after endocytosis.

P-418

Regulation of PIP2 clustering in neuron-like model cells by synaptic protein palmitoylation

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Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) is a minor component of the plasma membrane known to a critical agent in the regulation of synaptic transmission. Clustering of $PI(4,5)P_2$ in synaptic active zones is important for synaptic transmission. However, $PI(4,5)P_2$ does not spontaneously segregate in fluid lipid membranes and another mechanism must be responsible for the lateral segregation of this lipid in active zones. Clustering of $PI(4,5)P_2$ is expected to be associated with lipid-protein interactions and possibly partition towards lipid rafts in the plasma membrane. Here we analyze the influence of protein palmitoylation on the formation of $PI(4,5)P_2$ clusters and on synaptic protein-$PI(4,5)P_2$ interaction by means of Förster resonance energy transfer measurements by fluorescence lifetime imaging (FRET-FLIM) and FRET confocal microscopy.

P-419

Imaging SpoIIIE proteins during *Bacillus subtilis* sporulation by in-vivo PALM microscopy

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Under adverse conditions, *B. Subtilis* produces stable, dormant endospores to enhance its survival (Kay, 1968; Errington, 2003). During sporulation an entire chromosome is transferred into the forespore. This process starts by the formation of an asymmetrically located division septum that leads to the formation of two unequally sized compartments: a large mother-cell and a smaller forespore. The septum traps about 30% of the chromosome to be transferred into the forespore. The remaining ($\sim$3Mbp) are then translocated from the mother cell into the forespore by an active mechanism involving the SpoIIIE DNA translocase. The mechanisms of translocation, particularly the control of the directionality, still remains unknown and various models have been proposed so far.
Since each model predicts very different distribution of SpoIIIE proteins at the sporulation septum, we used PALM microscopy (photoactivated localization microscopy) to investigate proteins localization in live-sporulating bacteria. Using this technique, we showed that SpoIIIE proteins are forming a single tight focus at the septum with a characteristic size of around 30nm. More surprising, the focus is usually localized in the mother cell compartment and the

mean distance between the SpoIIIE focus and the septum is 35nm. Our data suggest that during the translocation process, SpoIIIE proteins are only forming stable complexes on the mother cell side, allowing then for a control of the chromosome translocation from the mother cell to the forespore.

P-420

Fast mobility of Bicoid captured by fluorescence correlation spectroscopy in live Drosophila embryos

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Morphogenetic gradients determine cell identity in a concentration-dependent manner and do so in a way that is both incredibly precise and remarkably robust. In order to understand how they achieve this feat, one needs to establish the sequence of molecular mechanisms starting with morphogen gradient formation and leading to the expression of downstream target genes. In fruit flies, the transcription factor Bicoid (Bcd) is a crucial morphogen that forms an exponential concentration gradient along the embryo AP axis and turns on cascades of target genes in distinct anterior domains. We measured Bcd-EGFP mobility in live *D. melanogaster* embryos using fluorescence correlation spectroscopy and fluorescence recovery after photobleaching. We found that Bcd-EGFP molecules had a diffusion coefficient on the order of $\sim 7\ \mu m^2/s$ during nuclear cycles 12-14, both in the cytoplasm and in nuclei. This value is large enough to explain the stable establishment of the Bcd gradient simply by diffusion. On the other hand, in the context of the extremely precise orchestration of the transcription of the *hunchback* Bcd target gene, it is too slow to explain how a precise reading of Bicoid concentration could be achieved at each interphase without the existence of a memorization process.

P-421

Single molecule studies of key processes during the initiation of innate and adaptive immune response

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The two pillars of the vertebrate immune system are the innate and adaptive immune response, which confer resistance to pathogens and play a role in numerous diseases. Here we exploit single molecule fluorescence imaging on live cells to study the key molecular processes that underpin these responses. The first project looks at the changes in the organisation of Toll-like receptor 4 (TLR4) on the cell surface of macrophages upon activation via Lipopolysaccharide (LPS), as it is currently not known whether a higher level of TLR4 organisation is required for the signalling process. Macrophages natively express TLR4 at a low level which allows for oligomerisation to be analysed in live cells by dynamic single molecule colocalisation (DySCo) using data obtained by total-internal reflection fluorescence (TIRF) microscopy. The experiments of the second project aim at determining the critical initial events in T-cell triggering by labelling key proteins like the TCR receptor and CD45 on the surface of live T-cells and following how their spatial distribution changes following the binding of the T-cell to a surface. This enables us to distinguish between the different models of T-cell triggering which are based on aggregation, segregation or a conformational change of the TCR.

P-423

The study of cells using scanning force microscopy

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The poster is focused on an interdisciplinary area of research concerning the surface properties of different cells, including pathogens. The use of Scanning Force Microscopy (SFM) to image and determine surface characteristics of *Chlamydia trachomatis* bacteria and two species each of *Rhodococcus* bacteria and *Leishmania* parasites will be discussed. Further to this, research into the adhesive properties of *Leishmania* promastigotes is proposed. This requires fabrication of different glycopolymer modified surfaces/tips to replicate some of the conditions experienced by the parasite whilst in the insect host. The prokaryotic and eukaryotic organisms considered have different impacts on the environment; *C. trachomatis* and *Leishmania* undergo complex multi-stage lifecycles and cause widespread human disease. This gives the research importance in a medical context, providing information that could be useful for future drug or vaccine research. The diverse species of genus *Rhodococcus* have potential for use as biofilms for environmental bioremediation.
The research should result in a combination of adhesion data and topographic images of the organisms, allowing accurate investigation of surface properties both in ambient conditions and when imaged in aqueous conditions such as under a saline buffer. Functionalization of SFM tips with hydrophilic or hydrophobic groups allows the study of interactions between the functional molecules and the surface of the organism, thus mapping the hydrophobicity of the organism's surface.

P-424

Tracing the microscopic motility of unicellular parasites

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The motility of unicellular parasites in mammalians seems very interesting, yet very complex. In a world, were inertia cannot be used for propulsion, in a world at low Reynolds

numbers, most of our everyday strategies of self-propulsion do not work.
One class of parasites that know their way around, the flagellate *Trypansome*, manage not only to survive in the blood stream, which is a lot faster than its own propulsion velocity and where the *Trypanosome* is constantly attacked by its host's immune response, but also to penetrate the blood-brain-barrier, which actually should be to tight to enter. Even though *Trypanosomes* are known for more than 100 years, their motility behaviour is not completely elucidated yet.
Now, using high-speed darkfield-microscopy in combination with optical tweezers in microfluidic devices and analyzing the recorded data, new light has been shed on the motility of these parasites. Astonishing results show that *Trypanosomes* are very well adapted to their hosts environment, they even can abuse red blood cells for their self-propulsion and use the bloodstream itself to drag antibodies bound to their surface to their cell mouth, where the antibodies are endocytosed and digested.

O-425
FRET approaches to signal transduction: from QD biosensors to live-cell EGFR signaling
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The first part of the presentation will discuss nanoparticle (quantum dot, QD) biosensors and nanoactuators that exploit novel and unusual FRET phenomena in the induction/detection of protein aggregation [1], reversible on-off QD photoswitching [2], and pH sensing [3]. The second part of the presentation will feature the application of an integrated chemical biological FRET approach for the *in situ* (in/on living cells) detection of conformational changes in the ectodomain of a receptor tyrosine kinase (the receptor for the growth factor EGF) induced by ligand binding [4]. The measurements were conducted with a two-photon scanning microscope equipped with TCSPC detection; novel methods for lifetime analysis ad interpretation were employed to confirm the concerted domain rearrangements predicted from X-ray crystallography.

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[2] Díaz S.A., Menéndez G.O., Etchehon M.H., Giordano L., Jovin T.M., and Jares-Erijman E.A. "Photoswitchable Water-Soluble Quantum Dots: pcFRET Based on Amphiphilic Photochromic Polymer Coating". *ACSNano* **5**:2795-2805 (2011).
[3] Menéndez G.O. et al., manuscript in preparation (2011).
[4] Ziomkiewicz I. et al., manuscript in preparation (2011).

P-426
Detecting protein conformational states in *C. elegans* in vivo by confocal fluorescence anisotropy
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The study of protein-protein interactions in vivo is often hindered by the limited acquisition speed of typical instrumentation used, for instance, for lifetime imaging microscopy. Anisotropy polarization is altered by the occurrence of Foerster Resonance Energy Transfer (FRET) and anisotropy imaging was shown to be comparatively fast and simple to implement. Here, we present the adaptation of a spinning disc confocal microscope for fluorescence anisotropy imaging that allowed to achieve in vivo imaging at high spatial and temporal resolution. We demonstrate the capabilities of this system and in-house developed analysis software by imaging living Caenorhabditis elegans expressing constitutive dimeric and monomeric proteins that are tagged with GFP.

O-427
Measuring intracellular viscosity: from molecular rotors to Photodynamic Therapy of cancer
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Viscosity is one of the main factors which influence diffusion in condensed media and is crucial for cell function. However, mapping viscosity on a single-cell scale is a challenge. We have imaged viscosity inside live cells using fluorescent probes, called molecular rotors, in which the speed of rotation about a sterically hindered bond is viscosity-dependent [1-3]. This approach enabled us to demonstrate that viscosity distribution in a cell is highly heterogeneous and that the local microviscosity in hydrophobic cell domains can be up to 100× higher than that of water. We demonstrated that the intracellular viscosity increases dramatically during light activated cancer treatment, called Photodynamic therapy (PDT) [2]. We also demonstrated that the ability of a fluorophore to induce apoptosis in cells during PDT [4], or to act as a benign molecular rotor, measuring viscosity, can be controlled by carefully selecting the excitation wavelength in viscous medium [5].

[1] M. K. Kuimova, et al *J. Amer. Chem. Soc.*, **2008**, *130*, 6672
[2] M. K. Kuimova, et al, *Nature Chem.*, **2009**, *1*, 69
[3] J. A. Levitt, et al *J. Phys. Chem. C*, **2009**, *113*, 11634
[4] H. A. Collins, *et al Nature Photonics*, **2008**, 2, 420-425
[5] M. K. Kuimova, et al *J. Amer. Chem. Soc.*, **2009**, *131*, 7948

P-428

Design of structured microenvironments for single cell transfection studies

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In the field of biophysics and nanomedicine, the cellular reaction and the kinetics of gene expression after transfection of live cells with plasmid DNA or gene-silencing siRNA is of great interest. In a previous study on the transfection kinetics of non-viral gene-transfer [1] we realised that the development of single-cell arrays would be a great step towards easy-to-analyse, high-throughput transfection studies. The regular arrangement of single cells would overcome the limitations in image-analysis that arise from whole populations of cells. In addition to that, the analysis of expression kinetics at the single-cell can help to identify the cell-to-cell variability within a cell population. In order to develop suitable single-cell arrays, we are currently adjusting the different parameters of such a microenvironment (e.g. size, shape, surface-functionalisation) in order to end up with a defined surrounding for single-cell transfection studies. In addition to that, we try to find the optimal uptake pathway for each of the different applications.

[1] G. Schwake et al., Biotechnol. and Bioeng. 105(4) (2010) 805.

P-429

Single molecule imaging of amyloid-peptide and the prion protein receptor on neurons

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The neurodegenerative disorder Alzheimer's disease (AD) causes cognitive impairment such as loss of episodic memory with ultimately fatal consequences. Accumulation and aggregation of two proteins in the brain - amyloid beta and tau - is a characteristic feature. These soluble proteins aggregate during the course of the disease and assemble into amyloid-like filaments. Recently it was found that the toxicity of soluble amyloid beta oligomers must also be taken into account for the pathogenesis of cognitive failure in AD. If oligomers are the predominant toxic species it would be pertinent to determine how they disrupt and impair neuronal function. The prion protein (PrP) receptor has been proposed to mediate amyloid beta binding to neuronal cells. We have characterised the interaction of the amyloid beta and the PrP receptor expressed on hippocampal and neuroblastoma cells at the single-molecule level. We do not detect any colocalisation of either the 40 or 42 amino acids variants with the PrP receptor.

O-430

SFM study of bacterial cell walls

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Bacterial biofilms are of the utmost importance in the study of environmental bioremediation and the design of materials for medical applications. The understanding of the mechanisms that govern cell adhesion must be analysed from the physics point of view in order to obtain quantitative descriptors.

The genus *Rhodococcus* is widely spread in natural environments. The species are metabolically diverse and thus they can degrade a wide range of pollutants. Due to their high hydrophobicity, these cells are very resistant to harsh conditions, are able to degrade hydrophobic substances (e.g. oil) and attach to high-contact angle surfaces. The hydrophobicity of several strains of *Rhodococcus* is measured and mapped using Chemical Force Microscopy (CFM) in the present study.

CFM relies on the functionalisation of Scanning Force Microscopy (SFM) tips using hydrophobic or hydrophilic groups. In CFM, the microscope is operated in the force-volume mode, which combines adhesion data with topographic images. The careful control of the tip chemistry permits the study of interactions between the functional groups on the tip and the bacterial surface, thus allowing the assessment of hydrophobicity.

In order to perform a CFM study, the cells need to be firmly anchored to a substrate under physiological conditions (i.e. under a nutrient media or a saline buffer). To this end, several adhesive surfaces have been tested in order to find the one that gives the best results.

P-431

Super-resolution microscopy in live-cell imaging

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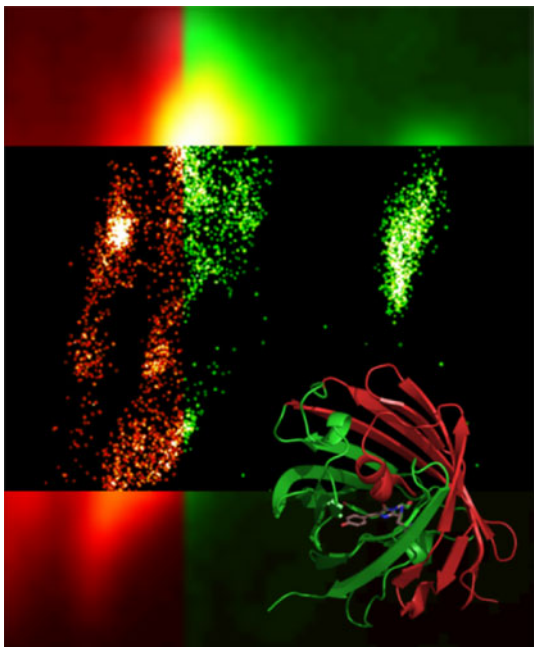
Optical microscopy is arguably the most important technique for the study of living systems because it allows 3D imaging of cells and tissues under physiological conditions under minimally invasive conditions. Conventional far-field microscopy is diffraction-limited; only structures larger than $\sim$200 nm can be resolved, which is insufficient for many applications. Recently, techniques featuring image resolutions down to $\sim$20 nm have been introduced such as localization

microscopy (PALM, STORM) and reversible saturable optical fluorescent transition microscopy (RESOLFT, STED). These methods are well suited for live-cell imaging and narrow the resolution gap between light and electron microscopy significantly.
We have used PALM imaging to study the formation and disassembly of focal adhesions of live HeLa cells in a high resolution pulse-chase experiment using monomeric IrisFP [1]. mIrisFP is a photoactivatable fluorescent protein that combines irreversible photoconversion from a green- to a red-emitting form with reversible photoswitching between a fluorescent and a nonfluorescent state in both forms. In our experiments a subpopulation of mIrisFP molecules is photoconverted to the red form by irradiating a specified region of the cell with a pulse of violet light. Migration of tagged proteins out of the conversion region can be studied by subsequently localizing the proteins in other regions of the cell by PALM imaging, now using the photoswitching capability of the red species. Real-time image reconstruction developed in our lab [2] allowed instant control imaging parameters.

[1] J. Fuchs, S. Böhme, S., F. Oswald, P. N. Hedde, M. Krause, J. Wiedenmann, and G. U. Nienhaus, *A Photoactivatable Marker Protein for Pulse-chase Imaging with Superresolution*, Nature Methods **7**, 627-630 (2010).
[2] P. N. Hedde, J. Fuchs, F. Oswald, J. Wiedenmann, and G. U. Nienhaus, *Online Image Analysis for Photoactivation Localization Microscopy,* Nature Methods **6**, 689-690 (2009).



P-432
Fluorescence live cell imaging of A549 cells in presence of PVP-Hypericin: Modifying excitation light dose of blue LED

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Live cell imaging of cancer cells is often used for in-vitro *studies* in connection with photodynamic diagnostic and therapy (PDD and PDT). Especially in presence of a photosensitizer, this live cell imaging can only be performed over relatively short duration (at most 1 hour). This restriction comes from the light-induced cell *damages (photodamages)* that result from rapid fluorescence photobleaching of photosensitizer. While these studies reveal exciting results, it takes several hours to discover the detailed effects of the photosensitizer on cell damage. Up to our knowledge, however, there is no general guideline for modification of excitation light dose to achieve that.
In this paper, the relation between excitation light doses, photobleaching of photosensitizer (PVP-Hyperycin) and cell vitality are investigated using human lung epithelial carcinoma cells (A549). The strategy of this paper is to reduce the excitation light dose by using a low-power pulsed blue LED such that the structures are visible in time-lapse images. Fluorescence signals and image quality are improved by labelling the cells with an additional non-toxic marker called carboxy-fluorescein-diacetate-succinimidyl-ester (CFSE). In total we collected 2700 time-lapse images (time intervals 2 min) of dual-marked A549 cells under three different light intensities (1.59, 6.34 and 14.27 mW/cm^2) and a variety of pulse lengths (0.127, 1.29, 13, 54.5 and 131 ms) over five hours. We have found that there is a nonlinear relationship between the amount of excitation light dose and cell vitality. Cells are healthy, i.e. they commence and complete mitosis, when exposed to low light intensities and brief pulses of light. Light intensities higher than 6.34 mW/cm^2 together with pulse durations longer than 13 ms often cause cell vesiculation, blebbing and apoptosis. In all other cases, however, we found no cell death. In the future, this striking nonlinearity will be studied in more detail.

P-433
Intranuclear visualization and distribution analysis of Pre Integration Complexes in MLV and HIV-1 infected cells

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Here we report for the first time the visualization of MLV viral particles in the nuclear compartment of infected cells. We

developed a fluorescent MLV virus by fusing GFP to the integrase (IN) of MLV. This system provides the possibility to efficiently detect MLV Pre Integration Complexes (PIC) inside the nuclei of infected cells. A detailed analysis of the spatial distribution of these fluorescently labelled PICs inside the nucleus has been performed: infected cells have been transfected with a fluorescent H2B histone or stained with epigenetic markers of heterochromatin (H3K9me3) or euchromatin (H4K16ac). Moreover, a comparative analysis was performed relatively to fluorescently labelled HIV-1 PICs (visualized by the trans-incorporation of IN-EGFP into the viral particle). The proposed system allows a detailed characterization of the intranuclear distribution of MLV fluorescent viral particles in infected cells and represents a powerful tool to investigate potential interactions between MLV PICs and cellular factors.

O-434
Multifunctional ion conductance microscopy for biophysical studies at nanoscale
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Progressive advances in scanning ion conductance microscopy (SICM)[1] enabled us to convert ordinary scanning probe microscope (SPM) in to versatile multifunctional technique. As an imaging tool, ion conductance microscopy is capable to deliver highest possible topographical resolution on living cell membranes among any other microscopy techniques[2]. Also, it can visualize surfaces complexity of those makes them impossible to image by other SPMs[3]. Ion conductance microscopy combined with a battery of powerful methods such as fluorescence resonance energy transfer (FRET) [4], patch-clamp, force mapping, localized drug delivery, nano-deposition and nano-sensing is unique among current imaging techniques. The rich combination of ion conductance imaging with other imaging techniques such as laser confocal and electrochemical [5] will facilitate the study of living cells and tissues at nanoscale.

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P-435
Fluorescence of living cells during senescence and stress
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Fluorescence of cells is an effective method which allows for defining changes in a set of cell metabolites initially, directly in living cells even at insignificant damage. Green plants emit a red fluorescence (RF, 630-700 nm), a far-red fluorescence (FRF, 700-800 nm), both from chlorophyll, and a blue-green fluorescence (BGF, 400-630 nm) vacuoles of epidermis that are related to secondary plant metabolism and UV- protection and piridine [NAD(P)H] and flavine nucleotides, the cofactors of metabolism. The emission spectrum depends on plant species, stage of development, anatomy and environmental factors experienced by the plant.
First leaves of wheat (*Triticum aestivum* L.), march pennywort (*Hydrocotyle vulgaris* L.) and coleoptiles of wheat (*Triticum aestivum* L.) seedlings, which were growing in light and dark conditions, were used to determine fluorescence of whole cells. Fluorescence emission spectrum was monitored by fluorescent microscopy using the spectrometer USB 4000. Fluorescence intensity F490, F680, F710 and F740 was determined and data was statistically analyzed in ANNOVA. We observed that BGF, RF and FRF intensity increased in the first leaves with the age of the seedlings. In the coleoptiles was observed great BGF intensity increase with the age of the seedlings. In the coleoptiles decreased RF intensity of the 144 and 196 hours old seedlings, and BGF intensity decreased of 196 hours old seedlings. It was found that emission spectrum and fluorescence intensity changes are induced by the lack of light and salt (*NaCl*) stress. Analysis of fluorescence spectrum can quickly and accurately indicate the outset of light and salt stress in plants.
There are analogical changes in fluorescence emission spectrum of plant cells in senescence and stress conditions. It was assumed that environmental stress and senescence have common mechanisms in plants. This changes can be monitored by fluorescent microscopy.

O-437
Triple-colour super-resolution imaging in living cells
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Super-resolution fluorescence imaging techniques based on single molecule localisation has opened tremendous

insight into the sub-micrometre organisation of the cell. Live cell imaging techniques such as fluorescence photoactivation localization microscopy (FPALM) are currently limited to dual-colour detection due to the restricted availability of red-fluorescent photoswitchable proteins. We employed photoswitching of the oxazine dye ATTO655 under reducing conditions for super-resolution imaging in the cytoplasm of living cells. For efficient and specific covalent labelling of target proteins, we have made use of the HaloTag system. ATTO655 was coupled to the HaloTag Ligand (HTL) and fast reaction of HTL-ATTO655 with the HaloTag enyzme was confirmed in *vitro* by solid phase binding assays. Efficient labelling of the membrane cytoskeleton using Lifeact fused to the HaloTag was observed and super-resolution imaging was readily achieved. Based on this approach, we managed to follow the nanoscale dynamics of the actin cytoskeleton as well as clathrin-coated pits using clathrin light chain fused to the HaloTag. We combined this technique with FPALM for triple-colour super-resolution imaging of the spatial distribution of membrane receptors in context of the membrane skeleton.

O-438

Simultaneous, multicolour single molecule imaging of the entire ErbB receptor family in live cells

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The ErbB family of receptor tyrosine kinases consists of four transmembrane proteins that transduce signals across the membrane to control cell fate. Growth factor binding results in homo- and hetero-interactions between these receptors at the membrane. ErbB receptors are implicated in many cancers, making them a target for therapeutic drugs. To date, studies of ErbB interactions have been limited to individual family members or specific pairs, giving an incomplete picture of the highly complex behaviour controlling positive and negative feedback loops and signalling outcomes.

To investigate ErbB receptor interactions, we have developed TIRF-based single molecule fluorescence microscopes capable of simultaneously imaging three, and soon five, fluorescence probes in live cells. We have also developed a catalogue of extrinsic fluorescent probes for 1:1 labelling of both endogenous and transfected ErbB family members in mammalian cells, plus a Bayesian approach to the analysis of single molecule data. This allows us to track active and inactive ErbB family members at the basal surface of a model breast cancer cell line that expresses physiological levels of all four receptors. We present here initial characterisation of the entire ErbB family together in the cell membrane.

P-439

Revealing the stoichiometry of endogenous G protein-coupled receptors (GPCRs) at the surface of live cells using single molecule imaging

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The human genome contains more than 800 G protein-coupled receptors (GPCRs); overall, 3-4% of the mammalian genome encodes these molecules. Processes controlled by GPCRs include neurotransmission, cellular metabolism, secretion, and immune responses. However it is the stoichiometry of these receptors that is the most controversial. The starting point for understanding GPCR function was the idea that these receptors are monomeric. On the other hand a lot of recent studies favour the concept that GPCR form dimers and are not capable of signalling as independent monomers. Recent single molecule studies try to solve this dilemma by suggesting that GPCRs form transient dimers with a lifetime of $\sim$100 ms. However questions remain about the physiological relevance of the preparations necessary for these studies, since they have not been performed on endogenous receptors. Here, we directly image individual endogenous receptors using an equimolar mixture of two colour fluorescent Fab fragments. We can then determine the receptors stoichiometry by quantifying its dynamic single molecule colocalisation (Dy-SCo) recorded by total-internal reflection fluorescence (TIRF) microscopy.

Trends in neutron scattering for biology

O-440

Function of phosphoglycerate kinase enabled by large domain movements

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The biological function of enzymes is often related to large-scale domain movements. Configurational changes are observed by methods like x-ray crystallography, which give a static image of the protein. Are these changes due to the substrate binding or are they also related to the crystal confinement, which favors specific configurations? In solution the protein can have a different configurations and undergo large scale fluctuations.

Phosphoglycerate kinase (PGK) has a widely open domain structure with a hinge near to the active center between the

two domains. The hypothesis of a substrate-induced configuration change, was first proposed by Banks et al. based on the comparison of crystal structures.
We have recently investigated the domain dynamics of PGK (1). Structural analysis by small angle neutron scattering revealed that the structure of the holoprotein in solution is more compact as compared to the crystal structure, but would not allow the functionally important phosphoryl transfer between the substrates, if the protein would be static. Brownian large scale domain fluctuations on a timescale of 50 ns was revealed by neutron spin echo spectroscopy. The observed dynamics shows that the protein has the flexibility to allow fluctuations and displacements that seem to enable function.

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O-441
Deciphering membrane insertion of the diphtheria toxin translocation domain
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Many physiological and pathological processes involve insertion and translocation of soluble proteins into and across biological membranes. However, the molecular mechanisms of protein membrane insertion and translocation remain poorly understood. Here, we describe the pH-dependent membrane insertion of the diphtheria toxin T domain in lipid bilayers by specular neutron reflectometry and solid-state NMR spectroscopy. We gained unprecedented structural resolution using contrast-variation techniques that allow us to propose a sequential model of the membrane-insertion process at angstrom resolution along the perpendicular axis of the membrane. At pH 6, the native tertiary structure of the T domain unfolds, allowing its binding to the membrane. The membrane-bound state is characterized by a localization of the C-terminal hydrophobic helices within the outer third of the cis fatty acyl-chain region, and these helices are oriented predominantly parallel to the plane of the membrane. In contrast, the amphiphilic N-terminal helices remain in the buffer, above the polar headgroups due to repulsive electrostatic interactions. At pH 4, repulsive interactions vanish; the N-terminal helices penetrate the headgroup region and are oriented parallel to the plane of the membrane. The C-terminal helices penetrate deeper into the bilayer and occupy about two thirds of the acyl-chain region. These helices do not adopt a transmembrane orientation. Interestingly, the T domain induces disorder in the surrounding phospholipids and creates a continuum of water molecules spanning the membrane. We propose that this local destabilization permeabilizes the lipid bilayer and facilitates the translocation of the catalytic domain across the membrane.

O-442
Fluorinated surfactants (FSs) for studying membrane proteins (MPs)
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The limited stability in vitro of MPs motivates the search of new surfactants (1-4). FSs with a polymeric hydrophilic head proved to be mild towards MPs (1).New FSs were designed with chemically defined polar heads for structural applications. Lac- derivative was efficient in keeping several MPs water soluble and active but formed elongated rods (2). The Glu- family was synthesized, characterized in by SANS and AUC and for its biochemical interest. The formation of rods is related to the low volumetric ratio between the polar head and hydrophobic tail. The surfactant bearing two Glucose moieties is the most promising one, leading to both homogeneous and stable complexes for both BR and the b_6f. It was also shown be of particular interest for the structural investigation of membrane proteins using SANS (3).

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O-444
Protein dynamics explored by Mössbauer effect and neutron scattering
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Protein functions are determined by structure and dynamics. The naked protein provides the structures that permit the motions necessary for the functions. The bulk solvent and the hydration shell are crucial for the control of the motions through producing two types of fluctuations, called α and β_h. The α fluctuations originate in the bulk solvent, affect the shape of the protein, and open channels. The β_h fluctuations originate in the protein's hydration shell, depend critically on hydration, but are independent of the solvent viscosity. They control internal motions[1]. To prove the connection between external fluctuations and protein motions tools are needed that measure fluctuations and motions externally and internally over broad ranges of time and temperature. The Mössbauer effect and quasielastic neutron scattering are such tools. In proteins both techniques show a rapid increase of the mean-square-displacement (msd) above about 180 K. The increase is accompanied by the appearance of a broad band. The standard explanation of the increase, dubbed "dynamical transition", is controversial and no satisfactory explanation for the broad band has been put forward. A new interpretation of the Mössbauer effect in proteins proves that there is no dynamical transition and that the separation into a sharp line and a broad band is misleading. The broad band is inhomogeneous and is composed of sharp lines that have been shifted by β_h fluctuations in the protein's

hydration shell and α fluctuations in the bulk solvent. In crystals the α fluctuations are absent. Using the dielectric spectrum of the β_h fluctuations, we predict the shape of the Mössbauer spectrum from 80 K to 295 K with one dimensionless coefficient. The explanation is important for the dynamics of proteins and for the interpretation of quasi-elastic neutron scattering in complex systems.

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P-445

The dynamic landscape in the multi-subunit protein, apoferritin, as probed by high energy resolution neutron spectroscopy

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By combining elastic and quasi-elastic neutron scattering data, and by applying theory originally developed to investigate dynamics in glassy polymers, we have shown that in lyophilised apoferritin above T $\sim$ 100 K the dynamic response observed in the pico- to nano-second time regime is driven by CH_3 dynamics *alone*, where the methyl species exhibit a distribution of activation energies. Our results suggest that over the temporal and spatial range studied the main apoferritin peptide chain remains rigid. Interestingly, similar results are reported for other smaller, more flexible lyophilised bio-materials. We believe this work elucidates fundamental aspects of the dynamic landscape in apoferritin which will aid development of complex molecular dynamic model simulations of super-molecules. A detailed appreciation of the relationships between dynamics and biological function will require analysis based on such models that realize the full complexity of macromolecular material.

P-446

Dynamics of DPPC vesicles in the presence of bioprotectants

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Biological systems must often be stored for extended periods of time. This is done by lyophilisation in the presence of lyoprotectants, such as sugars, which results in stable products at ambient conditions.[1] In an effort to understand the mechanism of preservation and stabilization, the interactions between sugars and liposome vesicles, which serve as a simple membrane model, have been studied extensively. Amongst the common sugars, trehalose has superior preservative effects [1] and accumulates to high concentrations in many anhydrobiotic organisms.

Despite many experimental and numerical studies three mechanisms are proposed: vitrification [2], preferential exclusion [3] and water replacement [4]. To gain more insight into the stabilization mechanism we have recently investigated the effect of trehalose on the bending elasticity of fully hydrated unilamellar vesicles of 1,2-dipalmitoyl-phosphatidylcholine (DPPC) in D_2O at temperatures below and above the lipid melting transition (Tm) using neutron spin-echo. The data was analyzed using the Zilman-Granek theory. At all temperatures measured, trehalose stiffens the bilayer suggesting strong interactions between trehalose and the lipid. Trehalose appears to broaden the melting transition but does not change the Tm. This agrees with observations using differential scanning calorimetry.

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O-447

Influence of macromolecular crowding on protein stability

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Cell interior is a complex environment filled with a variety of different objects with respect to shape and size. Macromolecules are present at a total concentration up to several hundred grams per litre and the overall occupied volume fraction can reach ö $\approx$ 0.3-0.4. Under crowding environment protein- protein interaction play a fundamental role. The crowding environment can affect some physical, chemical, and biological properties of biological macromolecules [1,2]. Traditionally, protein folding is studied *in vitro* at very low concentration of proteins. Under such conditions, small globular single chain proteins can unfold and refold quite rapidly depending mainly to the nature of the solvent. Such processes have been very intensively studied, since folding of proteins into their native structure is the mechanism, which transforms polypeptide into its biologically active structure. Protein misfolding is involved in a very high number of diseases [4] (*e.g.* Alzheimer, Parkinson, and Kreuzfeld-Jacob diseases, type II diabetes, …).

Theoretically, the problem was studied by the introduction of the concept of excluded volume [5]. In recent papers [6,7], Minton uses statistical thermodynamic models to address the question. He predicted that inert cosolutes *stabilize* the native state of proteins against unfolding state mainly by *destabilizing* the unfolded state and that the dimension of the unfolded state decreases with increasing the concentration of cosolute in a measurable way.

Small Angle Neutron Scattering (SANS) is a technique of choice for such study because, by using appropriate mixtures of light and heavy water, it is possible to match the scattering length density of the solvent to the one of the cosolute and thus to measure the conformation of a molecule at low concentration in a presence of a high concentration of another one.
We will present a complete experimental study of the mechanism that leads to protein stabilization by macromolecular crowding [7,8].

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P-448

Coupled dynamics of protein and hydration water studied by inelastic neutron scattering and molecular dynamics simulation

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Proteins work in an aqueous environment at ambient temperature. It is widely accepted that the proteins are flexible and mobile. The flexibility and mobility, that is, protein dynamics are essential for protein functions. Neutron incoherent scattering is one of the most powerful techniques to observe protein dynamics quantitatively.
Here I will talk about dynamics of protein and its hydration water. The structure of a soluble protein thermally fluctuates in the solvated environment of a living cell. Understanding the effects of hydration water on protein dynamics is essential to determine the molecular basis of life. However, the precise relationship between hydration water and protein dynamics is still unknown because hydration water is ubiquitously configured on the protein surface. We found that hydration level dependence of the onset of the protein dynamical transition is correlated with the hydration water network. Hydration water dynamics change above the threshold hydration level, and water dynamics control protein dynamics. These findings lead to the conclusion that the hydration water network formation is an essential property that activates the anharmonic motions of a protein, which are responsible for protein function.

O-449

Nanobiology: membranes and proteins in motion

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One of the major challenges of neutron scattering is to contribute to biology and life-sciences. Modern neutron facilities and in particular the upcoming European Spallation Source are premier research tools to tackle this important challenge. Molecular dynamics and interactions in membranes can be studied under physiological conditions using neutron beams. I will present exciting recent results and potential applications to demonstrate capabilities of present and future neutron instrumentation.

O-451

Thermal fluctuations of hemoglobin from different species

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Thermal motions and stability of hemoglobin of three endotherms (platypus - Ornithorhynchus anatinus, domestic chicken - Gallus gallus domesticus and human - Homo sapiens) and an ectotherm (salt water crocodile - Crocodylus porosus) were investigated using neutron scattering and circular dichroism. The results revealed a direct correlation between the dynamic parameters, melting -, and body temperatures. On one hand, a certain flexibility of the protein is mandatory for biological function and activity. On the other hand, intramolecular forces must be strong enough to stabilize the structure of the protein and to prevent unfolding. Our study presents experimental evidence which support the hypothesis that the specific amino acid composition of Hb has a significant influence on thermal fluctuations of the protein. The amino acid sequence of Hb seems to have evolved to permit an optimal flexibility of the protein at body temperature. Macromolecular resilience was found to increase with body and melting temperatures, thus regulating Hb dynamics.

Cytoskeleton and cell migration

P-452

Optical trapping microrheology in cultured human cells

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Introduction: We present the microrheological study of the two close human epithelial cell lines: non-malignant HCV29 and cancerous T24. The optical tweezers tracking was applied to extract the trajectories of endogenous lipid granules which were analyzed using a recently proposed equation for mean square displacement (MSD)[1].
Methods: The set-up is based on a Nd:YAG laser, with the wavelength of 1064 nm. The endogenous granules of diameter about 0.4 μm have been chosen as the microrheological tracers, and the tracer trajectory is recorded through the back focal plane interferometric position detection.
Results: Our 1MHz detection scheme has allowed to observe the subdiffusion inside the living cells for the lag times region 10^{-5}-10^{-3} s. At long time the MSD becomes influenced by the optical potential and tends to a constant. Recently, Desposito and Vinales have derived the analytical formula for the MSD in the case of subdiffusion influenced by an optical trap[1]:

$$\langle \Delta r^2(t) \rangle = \frac{2k_BT}{k} n\left(1 - E_\alpha\left(-\frac{k}{\gamma_\alpha}t^\alpha\right)\right), \quad (1)$$

Where k is the trap spring constant, α is the subdiffusion exponent and E_α is the Mittag-Leffler function. The parameters obtained by fitting this equation to the experimental MSDs are summarized in Table 1.
Discussion and Conclusions:
At short lag times we have not found any difference between the two cell types, contrarily to the previous results obtained by AFM[2]. For both cell lines the subdiffusion exponent, α was found close to ¾, the value predicted by the theory of semiflexible polymers. But the crossover frequency X_3, was found smaller for the cancerous cells for all datasets. It corresponds to passage to the confined regime at longer times. We attribute it to the bigger impact of molecular motors. The cancerous cells show the larger MSDs at long lag time, what confirms the literature results for other cell lines[3] and provides the explanation for the AFM results which have been obtained at low frequency.

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O-453

Dynamics of confined actin networks in microchambers

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We study the spatiotemporal evolution of fibrous protein networks present in the intracellular and extracellular matrices. Here, we focus on the *in vitro* actin network dynamics and evolution. In order to study the hierarchical self-assembly of the network formation in a confined environment and provide external stimuli affecting the system minimally, we introduce a new microfluidic design.
The microfluidic setup consists of a controlling channel to which microchambers of different shapes and sizes are connected through narrow channels. This design results in having mainly convective transport in the controlling channel and diffusive transport into the microchamber. Rhodamine labeled actin monomers diffuse into the chamber. After polymerization is induced, they form a confined entangled network. Cross-linking proteins can then be added to increase the network complexity. Moreover, we can generate gradients of reactants across the microchambers and

Table 1 Parameters *obtained from fitting Eq. (1) to the MSD of the granules inside cells. The uncertainty is the standard deviation of parameters between different granules*

Trap stiffness (N/m)	Cell line	$2k_BT/k$(nm^2)	α	$X_3 = \left(\frac{k}{\gamma_\alpha}\right)^{\frac{1}{\alpha}}$ (Hz)
$4{\cdot}10^{-6}$	HCV29	1277±721	0.70±0.07	11±9
	T24	1413±669	0.69±0.07	6±3
10^{-5}	HCV29	679±258	0.73±0.02	36±18
	T24	487±198	0.67±0.05	10±7
$3{\cdot}10^{-5}$	HCV29	186±58	0.73±0.04	172±88
	T24	218±52	0.75±0.03	166±103

observe the response of the actin networks. These networks are analyzed for various network properties such as length distribution of links, connectivity distribution of nodes as well as fluctuations of links and nodes.

P-454

Carbon nanotubes as mechanical probes of equilibrium and non-equilibrium cytoskeletal networks

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Mechanics of cells are determined by the filamentous protein networks of the cytoskeleton. The activity of motor proteins typically creates strong fluctuations that drive the system out of equilibrium. In order to characterize the networks one needs probes that ideally span the characteristic length scales between nanometers and micrometers. Single-walled carbon nanotubes (SWNTs) are nanometer-diameter filaments with tunable bending stiffness. On a Brownian energy scale they have persistence lengths of about 20 – 100 micrometers and show significant thermal fluctuations on the cellular scale of a few microns. Diffusive motion and local bending dynamics of SWNTs embedded in a polymer network reflect forces and fluctuations of the embedding medium. We study the motion of individual SWNTs in equilibrium and non-equilibrium networks by near infrared fluorescence microscopy. We show that SWNTs reptate in the network. We will discuss the possibility of relating the local dynamic behavior of SWNTs as multi-scale probes to the viscoelastic properties of the surrounding network.

P-456

Structural and mechanical properties of VASP-mediated f-actin bundle networks

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Vasodilator-stimulated phosphoprotein (VASP) is a crucial regulator of actin dynamics. It is important in cellular processes such as axon guidance and migration, promoting assembly of filopodia and lamellipodia. VASP's multiple domain structure increases the range of interactions it has with actin monomers, filaments, and other proteins and it displays multiple binding modes both *in vitro* and *in vivo*, including barbed end elongation and filament bundling. However, it is not fully understood how VASP affects the structural and mechanical properties of actin networks. We characterize VASP-mediated bundling of actin networks in a simplified *in vitro* system using confocal microscopy and quantify mechanical properties with rheology measurements. We show that the network properties differ from other actin bundling proteins and reflect VASP's multiple domain structure, displaying a complex bundling phase space that depends upon solution conditions. We observe the formation of large bundle aggregates accompanied by a reduction in network elasticity at high protein ratios. In addition, we change VASP's actin binding mode and eliminate bundling by introducing free actin monomers. Finally, we show preliminary results from a biomimetic system that extends the range of actin-VASP interaction.

P-458

Cell migration or proliferation? The go or grow hypothesis in cancer cell cultures

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Background: Cancer related death is constantly growing in the past decades. The mortality of solid tumors is mostly due to the metastatic potential of tumor cells which requires a fine adjustment between cell migration and cell proliferation. As the metabolic processes in the cell provide a limited amount of available energy (i.e. ATP) the various biological processes like cell motility or DNA synthesis compete for the ATP available. The go or grow hypothesis postulates that tumor cells show either high migration or proliferation potential. In our study we investigate on a large series of tumor cell lines whether this assumption stands for malignant cells.
Materials and methods: Twenty tumor cell lines derived from malignant mesothelioma (mesodermal origin) and malignant melanoma (neuroectodermal origin) were subjected to three-days-long time-lapse videomicroscopic recordings. Cell motility and proliferation were characterized by the probability of cell division within 24 hours and the 24-hour migration distance of the cells.
Results: We found a wide range in both the cell migratory activity and the proliferation capacity in our series. The 24-hour migration distance ranged from 40 to 300 micron and from 10 to 130 micron in mesothelioma and melanoma cells, respectively. The lowest 24-hour cell division probability was found to be 0.21 in both the melanoma and mesothelioma series while the highest proliferation activity reached 1.1 and 1.4 in melanoma and mesothelioma, respectively. Interestingly, in the melanoma cell lines we found a significant positive correlation (r=0,6909; p=0,0186) between cell proliferation and cell migration. In contrast our mesothelioma cell lines displayed no correlation between these two cellular processes.
Conclusions*:* In summary our findings demonstrate that the investigated tumor cells do not defer cell proliferation for cell migration. Important to note the tumor cells derived from various organ systems may differ in terms of regulation of cell migration and cell proliferation. Furthermore our observation is in line with the general observation of pathologists that the highly proliferative tumors often display significant invasion of the surrounding normal tissue.

O-459
Driving cytoskeletal remodeling by extracellular matrix mechanics
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Many cell types are sensitive to mechanical signals. One striking example is the modulation of cell proliferation, morphology, motility, and protein expression in response to substrate stiffness. Changing the elastic moduli of substrates alters the formation of focal adhesions, the formation of actin filament bundles, and the stability of intermediate filaments. The range of stiffness over which different primary cell types respond can vary over a wide range and generally reflects the elastic modulus of the tissue from which these cells were isolated. Mechanosensing also depends on the type of adhesion receptor by which the cell binds, and therefore on the molecular composition of the specific extracellular matrix. The viscoelastic properties of different extracellular matrices and cytoskeletal elements also influence the response of cells to mechanical signals, and the unusual non-linear elasticity of many biopolymer gels, characterized by strain-stiffening leads to novel mechanisms by which cells alter their stiffness by engagement of molecular motors that produce internal stresses. The molecular mechanisms by which cells detect substrate stiffness are largely uncharacterized, but simultaneous control of substrate stiffness and adhesive patterns suggests that stiffness sensing occurs on a length scale much larger than single molecular linkages and that the time needed for mechanosensing is on the order of a few seconds.

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P-460
Against the wall: microtubule dynamics is regulated by force and end-binding proteins
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Microtubule (MT) plus-end dynamics is essential for cell morphogenesis. It is regulated by specific end-binding proteins, as well as by forces generated when MTs grow against obstacles such as the cell cortex. However, it is largely unknown whether end-binding proteins qualitatively or quantitatively change the way MTs respond to force. We therefore investigate dynamic MTs in the presence of the fission yeast proteins Mal3, Tea2, and Tip1 in vitro. Head-on forces are created by growing the MTs against micro-fabricated rigid barriers. Using TIRF microscopy we show that the protein complex enhances MT growth velocity and induces catastrophes. Moreover, in presence of the proteins and a force catastrophes become even more frequent. The catastrophe time then shortens by nearly and order of magnitude. To improve our insight into the underlying mechanism we need to elucidate the growth velocities at the barrier with nanometer resolution. This we achieve with force-clamped optical tweezers.

P-461
Expression of cytoskeletal protein titin during adaptation to extreme conditions
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To explore potential role of cytoskeletal component in cardiomyocyte for adaptation to extreme conditions was carried out the comparative study of expression of cytoskeletal sarcomeric protein titin in myocardium of ground squirrels during hibernation and gerbils after spaceflight. We have revealed a two-fold increase in content of long N2BA titin isoform as compared to short N2B titin isoform in different heart chambers of hibernating ground squirrels. The prevalence of the long titin isoform is known to determine the larger extensibility of heart muscle that promotes, according to Frank-Starling law, the increase in force of heart contractility for pumping higher viscous blood during torpor and adapting the myocardium to greater mechanical loads during awakening. Moreover, titin mRNA level showed seasonal downregulation in which all hibernating stages differed significantly from summer active level. It is possible that the decline of mRNA and protein synthesis during hibernation may be regarded as the accommodation for minimization of energetic expenditures. We have not revealed differences in titin mRNA levels between control gerbils and gerbils after spaceflight. But we have also observed the two-fold growth in the amount of N2BA titin isoform in left ventricle of gerbils after spaceflight that is likely to be directed to the restoration of the reduced heart contractility at zero-gravity. These results suggest that the increase of the content of the long N2BA titin isoform may serve as universal adaptive mechanism for regulating of heart function in response to the extreme conditions.

P-462
Interaction of WH2 domain of ABBA with monomeric actin
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ABBA is a novel member of the I-BAR protein family. I-BAR proteins play an important role in the formation of membrane protrusions by deforming the plasma membrane and recruiting the components of the polymerising actin filament machinery. They share a similar domain organisation which constitutes the membrane and F-actin binding IMD domain and the G-actin interacting WH2 domain. The role of the WH2 motif of the I-BAR protein family is well characterized, however, the function of the WH2 motif of ABBA has remained elusive. In the

light of this consideration our research focused on the interaction of WH2 domain of ABBA with G-actin. The affinity of WH2 motif of ABBA to ADP- and ATP-actin monomer was investigated by applying fluorescence anisotropy measurements. It turned out that the WH2 domain preferentially bound to the polymerisation competent ATP-actin monomer. In kinetic fluorescence assays the WH2 domain slowed down the rate of nucleotide exchange on actin. This result was supported by fluorescence quenching experiments that revealed a closed conformation for the nucleotide binding cleft of actin in the presence of ABBA. Our findings suggest that the WH2 domain of ABBA behaves similarly to the typical WH2 domains, which means that it has a G-actin sequestering function.

O-463

Are biomechanical changes necessary for tumor progression?

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With an increasing knowledge in tumor biology an overwhelming complexity becomes obvious which roots in the diversity of tumors and their heterogeneous molecular composition. Nevertheless in all solid tumors malignant neoplasia, i.e. uncontrolled growth, invasion of adjacent tissues, and metastasis, occurs. Physics sheds some new light on cancer by approaching this problem from a functional, materials perspective. Recent results indicate that all three pathomechanisms require changes in the active and passive cellular biomechanics. Malignant transformation causes cell softening for small deformations which correlates with an increased rate of proliferation and faster cell migration. The tumor cell's ability to strain harden permits tumor growth against a rigid tissue environment. A highly mechanosensitive, enhanced cell contractility is a prerequisite that tumor cells can cross its tumor boundaries and that this cells can migrate through the extracellular matrix. Insights into the biomechanical changes during tumor progression may lead to selective treatments by altering cell mechanics. Such drugs would not cure by killing cancer cells, but slow down tumor progression with only mild side effects and thus may be an option for older and frail patients.

P-464

Investigating nuclear migration with the help of micropatterned surfaces

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Nuclear migration is a general term for a non-random movement of the nucleus toward specific sites in the cell. This phenomenon has been described throughout the eukaryotes from yeast to mammals. The process is however still poorly understood in mammalian cells. By using microcontact printing we are able to regulate the geometry and spreading of cultured cells. Adhesive micropatterns of fibronectin provide an attachment surface for the cells whereas the passivation of the surface by PLL-PEG prevents protein, thus cell adhesion. Live cell imaging by time-lapse microscopy has shown that under these conditions cells gain a bipolar shape, and more interestingly, the nuclei of the cells showed auto-reversed motion. Our research tries to understand the molecular cues and mechanisms behind the observed cellular and nuclear movement. We have already shown that the cytoskeleton plays an important role in this phenomena but the exact players and the detailed mechanism remain to be clarified. In order to identify the most important components and their relationship have drug treatments and siRNA experiments have been applied. Although our research focuses mainly on the motility of the nucleus, it may also help to get a better understanding of the general theme of cell migration.

P-466

Modulation of cell internal tension in response to external geometrical adhesive cues

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Mechanotransduction is one of the recent area that brings attention of both physicists, chemists and biologists. Recent progress at the interface between those fields have shown that external physical cues can strongly affect cell behavior. Thus, it has been demonstrated that external physical signals such as substrate rigidity can have dramatic impacts on cellular functions such as migration, adhesion, division and also cell differentiation (*A. Engler et all Cell* ***126****: 677-689 (2006)*.).

Cells have the ability, *via* their cytoskeleton (cell internal structure), to exert traction forces on their micro-environment. These forces are transmitted to cell environment (substrate or neighboring cells) through adhesive contacts. Reversely cells are also submitted to forces from their environment (breathing, muscle contraction etc etc…) Many tools have been developed in order to elucidate this bidirectional biomechanical interactions that has been shown to be fundamental for tissue homeostasis. However current techniques have many limitations related to their difficulties to obtain statistical quantification regarding cell biomechanical activity.

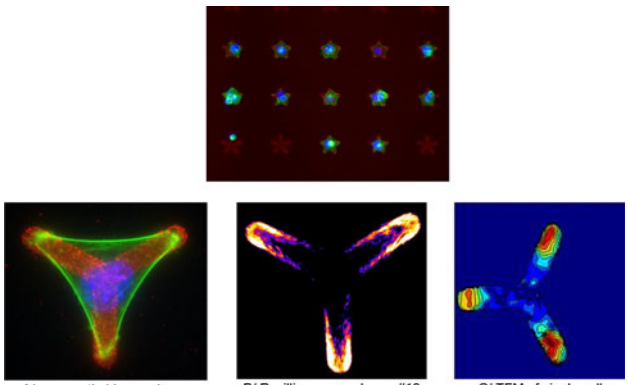
Contrary to classical biological approaches, here I present a novel technique that enable us to quantify cell forces that cell exert on standardized micro-environments. Briefly, by using a combination of Fast Fourier Traction force cytometry (Butler et al. Am. J. Physiol. Cell Physiol. 2002) and Micropatterning Technique (ChristopherChen et al.,*Biotechnol. Prog.* **1998,**) on soft hydrogel we are able to create regular array of cells for which architecture is controlled at the single cell level. Finally, as our micro-chips are designed on soft substrates we are able to correlate these architecture to the forces developed by the cells.

A/green:actin,blue:nucleus, B/ Paxillin averaged over #19 C/ TFM of single cell

red:pattern

single cell on Tripod shape;

To conclude, my goal is to correlate absolute quantified force with cytoskeletal organization.



P-467
Simultaneous quantification of cellular motility, redox status and membrane fluidity
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Cell motility involves a number of strategies that cells use to move in their environments in order to seek nutrients, escape danger and fulfil morphogenetic roles. When these processes become uncontrolled, pathological behaviours, like cancer or metastasis of cancerous cells, can occur. Here we present a new method for the contextual quantification of cellular motility, membrane fluidity and intracellular redox state, by using the ratiometric, redox-sensitive protein rxYFP and the ratiometric fluidity-sensitive probe laurdan. We provide evidence that dynamic redox and fluidity changes are correlated with signaling processes involved in cellular motility. These findings may pave the way to novel approaches for the pharmacological control of cell invasiveness and metastasis.

P-468
Manipulation of cellular mechanics
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Rheological properties of cells determined by the underlying cytoskeleton (cortex) are key features in cellular processes like cell migration, cell division, and cell morphology. Today it is possible to investigate local cellular elastic properties under almost physiological conditions using the AFM. By performing force indentation curves on local areas on a cell surface the use of contact mechanic models provides the Young's modulus, comprising information about the elastic properties of cells. The administration of cytoskeleton modifying substances into the cell is achieved by microinjection.

We are also investigating morphological changes and rearrangements of the cytoskeleton in time resolved impedance measurements. Electric cell-substrate impedance sensing is a label-free and minimal invasive technique which allows monitoring morphological changes of cells in real time. Readout of the impedance is sensitive to changes in cell-substrate contacts as well as density of cell-cell contacts yielding important information about the integrity of the cell layer and changes in the properties of the cell membrane. We are studying the cellular response to modification of the cytoskeleton e.g. by introducing proteins which affect directly the organization of the actin structure like ezrin.

P-469
Mechanical characterization of actin gels by a magnetic colloids technique
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The actin polymer is central in cell biology: it is a major component of cytoskeleton and it plays a fundamental role in motility, division, mechanotransduction…. Its polymerization just beneath the cell membrane generates forces responsible for cell movement. Actin filaments form a network whose architecture is defined by the nature of the binding proteins and depends on the location in the cell. For example, in the structure which leads cell migration, the lamellipodium, the gel is branched due to its interaction with Arp2/3 protein complex. Determining the mechanical properties of such actin network is a crucial interest to understand how forces are generated and transmitted in living cells. We grow a branched actin network from the surface of colloids using the Arp2/3 machinery. The particles are super paramagnetic and they attract each other via dipole-dipole interaction to form chain. By increasing the magnetic field we apply an increasing force to the gel and we optically measure the resulting deformation. From those measurements we deduce a Young modulus for a large amount of data. We are characterizing different networks by varying the concentration of the capping and branching proteins and we show how mechanics can be regulated by the different proteins.

O-470
Automated image analysis of microtubule dynamics and maintenance of fission yeast cells morphology
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Microtubules (MTs) are central to the organization of the eukaryotic intracellular space and are involved in the control of cell morphology. In fission yeasts cells MTs transport polarity factors to poles where growth is located, thus ensuring the establishment and maintenance of the characteristic spherocylindrical shape. For this purpose, MT polymerization dynamics is tightly regulated. Using automated image

analysis software, we investigated the spatial dependence of MT dynamics in interphase fission yeast cells. We evidenced that compressive forces generated by MTs growing against the cell pole locally reduce MT growth velocities and enhance catastrophe frequencies. In addition, our systematic and quantitative analysis (in combination with genetic modifications) provides a tool to study the role of +TIPs (plus-end tracking proteins) such as Mal3 and Tip1 in the spatial regulation of MT dynamics. We further use this system to decipher how the linear transport by MT interferes with the feedback circuitry that assures the correct spatial distribution of Tea1, the main polarity factor in fission yeast cells.

P-471
Modeling of actin cytoskeleton inside giant unilamellar vesicles
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Actin is one of the most abundant proteins which forms the highly dynamic cytoskeleton in all eukaryotic cells. It is essential for cell movement processes and also provides the cell membrane with high mechanical strength. For a better understanding of the actin cytoskeleton function as a stabilizing but also flexible 3D-network in living cells it is adjuvant to have a suitable model system.
For modeling the complex cytoskeleton in a bottom up approach, we use giant unilamellar vesicles created of artificial lipid bilayers to simulate the cell membrane and encapsulate monomer actin inside the vesicle aqueous core. Afterwards by ion controlled actin polymerization which is located at the inner leaflet of the vesicles, we induce a change of shape and rigidity of the liposomes.

O-472
Control of the fast, slow and reverse gear of the yeast mitotic kinesin-5 Cin8
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The dynamics of the cytoskeleton are largely driven by cytoskeletal motor proteins. Complex cellular functions, such as mitotsis, need a high degree of control of these motors. The versatility and sophistication of biological nanomachines still challenges our understanding. Kinesin-5 motors fulfill essential roles in mitotic spindle morphogenesis and dynamics and were thought to be slow, processive microtubule (MT)-plus-end directed motors. Here we have examined *in vitro* and *in vivo* functions of the *Saccharomyces cerevisiae* kinesin-5 Cin8 using single-molecule motility assays and single-molecule fluorescence microscopy. *In vivo*, the majority of Cin8 motors moved slowly towards MT plus-ends, but we also observed occasional minus-end directed motility episodes. *In vitro*, individual Cin8 motors could be switched by ionic conditions from rapid (up to 50 μm/min) and processive minus-end, to bidirectional, to slow plus-end motion. Deletion of the uniquely large insert in loop 8 of Cin8 induced bias towards minus-end motility and strongly affected the directional switching of Cin8 both *in vivo* and *in vitro*. The entirely unexpected *in vivo* and *in vitro* switching of Cin8 directionality and speed demonstrate that kinesins are much more complex than thought. These results will force us to rethink molecular models of motor function and will move the regulation of motors into the limelight as pivotal for understanding cytoskeleton-based machineries.

P-473
Morphological and dynamical changes during TGF-β induced epithelial-to-mesenchymal transition
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The epithelial-to-mesenchymal transition (EMT) is a program of cellular development associated with loss of cell-cell contacts, a decreased cell adhesion and substantial morphological changes. Besides its importance for numerous developmental processes like embryogenesis, EMT has also been held responsible for the development and progression of tumors and formation of metastases. The influence of the cytokine transforming growth factor 1 (TGF-β1) induced EMT on structure, migration, cytoskeletal dynamics and long-term correlations of the mammalian epithelial cell lines NMuMG, A549 and MDA-MB231 was investigated by time-resolved impedance analysis and atomic force microscopy (AFM) performing force-indentation measurements. The three cell lines display important differences in cellular morphology mirrored in changes of their elastic response (Young modulus), as well as their dynamics upon TGF-β1 treatment.
Impedance based measurements of *micromotility* reveal a complex dynamic response to TGF-β1 exposure which leads to a transient increase in fluctuation amplitude and long-term correlation. Additionally, the investigation of cellular elasticity via AFM depicts the different cytoskeletal alterations depending on the metastatic potential of the used cell type.

P-474
Physics of cellular mechanosensitivity studied with biomimetic actin-filled liposomes
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Biological cells actively probe the mechanical properties of their tissue environment by exerting contractile forces on it,

and use this information to decide whether to grow, migrate, or proliferate. The physical basis for cell contractility is the actin cytoskeleton, which transmits motor generated stresses to mechanosensitive adhesions sites that anchor the cell to the tissue. The origins of mechanosensing are far from understood due to the complex interplay of mechanical effects and biochemical signaling that occurs far from equilibrium. We use a quantitative biophysical approach based on biomimetic constructs to elucidate physical principles that underlie active mechanosensing in biological cells. We have built realistic *in vitro* models of contractile cells by encapsulating cross-linked actin networks together with myosin motors in cell-sized membranous containers (liposomes). Our method has several advantages over prior methods, including high liposome yield, compatibility with physiological buffers, and chemical control over protein/lipid coupling. I will show contour fluctuation spectra of constructs and first data on mechanical response obtained by laser tweezers microrheology. Our work will yield novel insights into stress generation and stiffness sensing of cells.

P-475
Setting up a system to reconstitute cytoskeleton-based protein delivery and patterning in vitro
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Keywords: Microtubules, fission yeast, cell polarity, protein patterning, plus end binding proteins.
Many different cell types, from mobile fibroblasts [1] to fission yeast cells [2], display non-homogenous protein patterns on their cell cortex. In fission yeast the cell-end marker protein Tea1 that among others is responsible for recruiting the actin dependent cell-growth machinery, is specifically located at the growing cell ends. Tea1 travels at the tips of growing microtubules and is delivered to the cell ends [2]. We aim to *in vitro* reconstitute a minimal microtubule plus-end tracking system that leads to cortical protein patterning in functionalized microfabricated chambers. Our model will allow us to perturb microtubule-based transport and diffusion independently and evaluate the resulting protein patterns.

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P-476
Effects of the neuronal Br-3 tropomyosin isoform on the actin dynamics
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The tropomyosins (TM) are dimeric actin-binding proteins that form longitudinal polymers along the actin filament groove. There is a great variety of isoforms, but the division of labour between the individual TMs and their significance is poorly understood. As in most cell types, also in the neurons several isoforms are present, whose spatio-temporal localisation is differentially regulated. The neuron-specific brain 3 isoform (TMBr-3) can be found in the axon of the mature cells. We aimed to clone, express and charaterise this protein in terms of its effects on the kinetic parameters of the actin filament. Using a pET28a construct we purified native, tag-free protein, and examined if it influences the rate of actin polymerisation or the stability of the filaments in the presence of either gelsolin or latrunculin-A, two depolymerising agents. In cosedimentation experiments the affinity of TMBr-3 to actin was $\sim$ 3μM, about six times that of skeletal muscle tropomyosin. The net rate of actin polymerisation was reduced by 17% in the presence of TMBr-3. The depolymerisation induced by gelsolin or latrunculin-A was inhibited in a concentration-dependent manner. TMBr-3 seems to stabilise actin filaments against disassembly without significant effect on the net polymerisation.

P-477
Cell mobility and metastatic spreading: a study on human neoplastic cells using optical tweezers
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The primary causes of death in cancer patients are local invasion and metastasis but their mechanisms are not yet completely understood. Metastatization is accompanied by alterations of the cytoskeleton and membrane structure leading to changes in their biomechanical properties*[1]*. In this study we analyzed by means of Optical Tweezers the mechanical properties of two different breast adenocarcinoma cell lines corresponding to different metastatic potential. OT were used to grab the plasma membrane by a 1,5 um silica bead and form a plasma membrane tether. We measured the force exerted by the cell membrane on the bead and drew the force-elongation curves. Fitting data in the Kelvin Body model *[2]*we found out the values for the viscoelastic parameters influencing the pulling of the membrane tethers.
The first cell line analyzed, MCF-7, associated to a low metastatic potential showed tether stiffness of 153 pN/um in average.
The second cell line, MDA-MB 231, poorly differentiated with a high metastatic potential had a tether stiffness of 36pN/um in average, that is a four times lower value. These results seems to confirm the hypotesis that metastasis prone cells are softer than less aggressive cancer cells, and support the use of OT for these measurements for its sub-pN force resolution and because cells are manipulated without damage.

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P-478
Moonlighting functions of TPPP/p25
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Tubulin Polymerization Promoting Protein (TPPP/p25) is a brain-specific protein that primary targets the microtubule network modulating its dynamics and stability. TPPP/p25 is a disordered protein with extended unstructured segments localized at the N- and C-terminals straddling a flexible region. TPPP/p25 is primarily expressed in oligodendrocytes where its multifunctional features such as tubulin polymerization promoting and microtubule bundling activities are crucial for the development of the projections in the course of oligodendrocyte differentiation enabling the ensheathment of axons with a myelin sheath that is indispensable for the normal function of the central nervous system.
Microtubule network, a major constituent of the cytoskeleton, displays multiple physiological and pathological functions in eukaryotic cells. The distinct functions of the microtubular structures are attained by static and dynamic associations of macromolecules and ligands as well as by post-translational modifications. TPPP/p25 is actively involved in the regulation of microtubule dynamics not exclusively by its bundling activity, but also by its tubulin acetylation-promoting activity. Atypical histone deacetylases, such as NAD-dependent SIRT2 and histone deacetylase-6, function outside of the nucleus and control the acetylation level of cytosolic proteins, such as tubulin. Acetylation-driven regulation of the microtubule network during cellular differentiation is an ambiguous issue. TPPP/p25 has been recently identified as an interacting partner and inhibitor of these deacetylases and their interaction decreased the growth velocity of the microtubule plus ends and the motility of the cells. We have established cell models for the quantification of the acetylation degree of microtubule network in correlation with its dynamics and stability as well as in relation to aggresome formation, that mimics the pathological inclusion formation.
The intracellular level of TPPP/p25 is controlled at post-transcription level by microRNA and at protein level by the proteosome machinery. Under pathological circumstances this disordered protein displays additional moonlighting function that is independent of its association with microtubule system or deacetylases; it enters aberrant protein-protein interaction with α-synuclein forming toxic aggregates within the neuronal and glial cells leading to the formation of inclusions characteristic for Parkinson's disease and multiple system atrophy, respectively.

P-479
Encapsulating active actomyosin networks in giant liposomes
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Keywords: Biomimetic systems, actin cytoskeleton, confinement, giant unilamellar liposomes, actomyosin network, membrane

The cell membrane separates the intracellular from the extracellular environment while intimately interacting with the cytoskeleton in numerous cellular functions, including cell division and motility. Cell shape changes are for a large part mediated by the contractile actomyosin network forming the cortex underneath the cell membrane. To uncover molecular mechanisms of cell shape control based on actin-membrane interactions, we built a novel biomimetic model system: a cell-sized liposome encapsulating an actively contracting actin-myosin network. Our fabrication method is inspired by a recent report of liposome preparation by swelling of lipid layers in agarose hydrogel films.[1] We extensively characterize important liposomal properties, finding diameters between 7 and 20 μm, unilamellarity, and excellent and uniform encapsulation efficiency. We further demonstrate chemical control of actin network anchoring to the membrane. The resulting liposomes allow quantitative tests of physical models of cell shape generation and mechanics.

1. Horger, K. S.; Estes, D. J.; Capone, R.; Mayer, M., Films of agarose enable rapid formation of giant liposomes in solutions of physiologic ionic strength. *J Am Chem Soc* **2009,** *131* (5), 1810-9.

P-480
The effect of mDia1-FH2 on the ATPase activity of actin
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Formins are conservative proteins with important roles in the regulation of the actin based microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD. In the interaction between actin and formin the FH2 domain plays a key role. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. These mDia1 formin fragments affect the conformation of the actin filaments in a concentration dependent manner. In the current work we have investigated whether the mDia1-FH2 affects the nucleotide exchange on the actin filaments. Steady-state fluorescence anisotropy and photometric coupled assay measurements showed that the ATP-ADP conversion was accelerated in the presence of formins, and the effect was stronger at greater formin concentrations. These observations indicate that there must be a tight coupling between the rate of nucleotide exchange on actin protomers and the conformational properties of the filaments.

O-481
Tropomyosin isoform specific regulation of nucleation factors
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In the cohesive structure of the cytoskeleton functionally distinct actin arrays orchestrate fundamental cell functions in a spatiotemporally controlled manner. Emerging evidences

emphasize that protein isoforms are essential for the functional polymorphism of the actin cytoskeleton. The generation of diverse actin networks is catalyzed by different nucleation factors, like formins and Arp2/3 complex. These actin arrays also exhibit qualitative and quantitative differences in the associated tropomyosin (Tm) isoforms. How the molecular composition and the function of actin networks are coupled is not completely understood.
We investigated the effects of different Tm isoforms (skeletal muscle, cytoskeletal 5NM1 and Br3) on the activity of mDia1 formin and Arp2/3 complex using fluorescence spectroscopic approaches. The results show that the studied Tm isoforms have different effects on the mDia1-, and Arp2/3 complex-mediated actin assembly. The activity of the Arp2/3 complex is inhibited by skTm and Tm5NM1, while TmBr3 does not have any effect. All three Tm isoforms inhibited the activity of mDia1. These results contribute to the understanding of the mechanisms by which tropomyosin isoforms regulate the functional diversity of the actin cytoskeleton.

P-482
Effect of collagen type I on human pulmonary artery endothelial cells
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Chronic thromboembolic pulmonary hypertension (CTEPH) is a dual pulmonary vascular disorder, which combines major vascular remodelling with small-vessel arteriopathy. The presence of fibroblasts in the clot, occluding the pulmonary arteries, and its composition create a microenvironment with increased collagen level, which might affect the local endothelial function. In this study, human pulmonary artery endothelial cells (hPAEC) were exposed to collagen type I to address the effect of the thrombotic microenvironment on the vessel wall forming cells.
The hPAECs, cultured under standard conditions were treated with 1, 10 and 100μg/ml of collagen type I for 6h and 24h. The changes in the endothelial cell barrier function were investigated by performing permeability and migration test as well as VE-cadherin staining.
Collagen type I treatment led to a decrease in VE-cadherin signal in hPAEC. The loosening of cell-cell contacts could be proven with a significant increase in permeability after 6h of collagen treatment with different concentrations. Besides the loosening of the cell-cell contacts, the hPAEC migration was also dose dependently retarded by collagen application over time.
Our data show that collagen-rich microenvironment leads to a disruption of the junctional proteins in hPAECs, indicating an environmental induced possible alteration in the function of endothelial cells in the clots of CTEPH patients.

Ion channels: structure and function

O-483
Targeting the promiscuous voltage sensor of Kv7.2 and TRPV1 channels by non-toxin gating-modifiers
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The pore and gate regions of voltage-gated cation channels have been often targeted with drugs acting as channel modulators. In contrast, the voltage sensing domain (VSD) was practically not exploited for therapeutic purposes, though it is the target of gating-modifier toxins. We recently designed novel diphenylamine carboxylates that are powerful Kv7.2 channel openers or blockers. Here we show that NH17 and NH29, two new Kv7.2 channel blocker and opener, respectively, act as gating modifiers. Mutagenesis and modeling data suggest that in Kv7.2, NH29 docks to the external groove formed by the interface of helices S1, S2 and S4 in a way, which stabilizes the interaction between two conserved charged residues in S2, and S4, known to interact electrostatically, in the open state of Kv channels. Interestingly, NH29 is also a potent blocker of TRPV1 channels, while the Kv7.2 channel blocker, NH17, activates TRPV1 and sensitizes the TRPV1 current activated by capsaicin. Our data suggest that the VSD of TRPV1 is also important for the effects of NH29 and NH17. Thus, subtle modifications in the VSD or in the chemical structure of the molecule drastically change the attributes of the gating-modifier, thereby stabilizing the channel in either the closed or the open state.

P-484
Investigation of Channelrhodopsin-2 mutants using resonance Raman spectroscopy
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Channelrhodopsin-2 (ChR2), a membrane-bound cation channel, is one of the proteins involved in the phototactic response of the alga *Chlamydomonas reinhardii*. Using resonance Raman spectroscopy, we have investigated the retinal chromophore that is covalently linked via a Schiff base to the ChR2 protein. The chromophore of the ChR2 H134R dark state was studied using low laser power and rotating

cuvette under mixing. With increasing laser power, accumulation of the deprotonated state P390 (M-state) in the laser spot was noted but without any significant increase of other intermediates. The resonance Raman spectra of the dark state displays symmetric band shapes in the C=C and C=N stretching region indicating the occurrence of only one isomeric form. The channel activity of ChR2 is most likely induced by the *all-trans* to *13-cis* isomerisation of the retinal chromophore, as judged from the similar spectral changes that occur during transition from dark state to P390 in ChR2 and from dark state to M412 in Bacteriorhodopsin. Additionally, we have found that the inactivation of the slow mutant ChR2 C128T is probably caused by hydrolysis of the retinal Schiff base creating a free retinal, due to the almost identical spectra of the side-chain intermediate and *all-trans* retinal in CCl_4.

P-485

Architecture of human perforin pore in pure lipid bilayers

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Perforin (PFN) is a component of the human innate immune system, is released by lymphocytes and its key function is to engineer the delivery of pro-apoptotic granzymes to the target cell cytoplasm, leading to a controlled suicidal cell death. To date, it has been unclear whether granzyme delivery is allowed through the aperture of pores in the cell membrane, or by some other means. Nevertheless, the ability of PFN to perforate the target membranes by stable pores is central to each proposed model. Here we present the characterization of functional PFN pores using single-channel conductance and fluorescence microscopy. We show that it forms pores heterogeneous in size (up to 20 nm) in planar lipid membranes (PLM) and unilamellar vesicles. From PLM experiments we observed that the opening step and the pore size are dependent on membrane lipid composition: disordered membranes favored gradual openings of the pore, while in ordered membranes pores opened in a discrete manner.
PFN seems capable of triggering an endocytosis-like event in addition to pore formation, suggesting a new paradigm for its role in delivering granzymes into target cells where they induce apoptosis.

O-486

Over-expression of human ion channels and transporters for structural studies

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The implementation of miniaturisation and high throughput screening has quickened the pace of protein structure determination. However, for most proteins the process still requires milligram quantities of protein with purity > 95 %. These amounts are required as a result of unavoidable losses during purification and for the extensive screening of crystallisation space. For integral membrane proteins (IMPs), one of the initial steps in the structure determination procedure is still a major bottleneck - the over-expression of the target protein in the milligram quantity range. With a view of developing guidelines for over-expression of human IMPs, a systematic approach using the three most common laboratory expression systems (*E. coli*, *S. cerevisiae*, Sf9 insect cells) was implemented. Initial expression levels were determined by either partial purification using Ni^{2+}-NTA (*E. coli*), green fluorescence protein (GFP) fluorescence using a C-terminal GFP tagged protein (*S. cerevisiae*) or FLAG tagged partial purification (Sf9 cells). The results show that *E. coli* is suitable for the over-expression of human IMPs in the required quantity range however protein size and complexity is an important factor. The yeast system is fast and affordable but, for the group of human IMPs tested, the expression levels were borderline. Finally for the insect cell system, the timelines are slower and it is in comparison costly to run, however, it can produce relatively large quantities of human IMPs.

P-487

Dipole modifiers affect properties of syringomycin channels in the presence of large organic anions

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An antifungal cyclic lipopeptide syringomycin E (SRE) from *Pseudomonas syringae* forms predominantly anion selective voltage-gated channels in membranes bathed in solutions of alkaline metal chlorides [Malev at al., Advances in planar lipid bilayers and liposomes, V. 8, 2008]. Effects of the membrane dipole potential (V_d) on the single channel conductance (G) and the steady-state number of open SRE-channels (N) in phosphocholine bilayers bathed in 0.4 M NaAsp and NaGlc (pH 6) were studied. Values of V_d were varied with introduction into membrane bathing solutions of either phloretin reducing V_d, or RH 421 increasing V_d. We found that G did not depend on V_d. This fact resulted from a loss of SRE pore anionic selectivity. A decrease of V_d caused an increase of N. At the same time, the observed changes in N were one order smaller than those of systems containing NaCl [Ostroumova et al., Langmuir, 2008]. Such difference in N values was assigned to specific adsorption of large anions on the bilayer/solution interface. The work was supported in part by RFBR (# 09-04-00883), SS-3796.2010.4, the Program of the RAS «MCB» and RSC # P1372 (MES, FTP<<SSEPIR>>).

P-488

Spectroscopic investigation of the structure and function of the copper ATPase CopB of *Enterococcus hirae*

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The Cu+-ATPase CopB of Enterococcus hirae is a bacterial P-type ATPase involved in resistance to high levels of environmental copper by expelling excess copper. The membrane protein CopB was purified from an over-expressing strain and solubilized in dodecyl-maltoside. By UV circular dichroism the secondary structure is predicted to contain 40-50 % α-helices and 10-15% β-sheets in agreement with estimates based on homology with the Ca ATPase SERCA1. We present CD-spectroscopic data on thermal unfolding of the protein to address the influence of the binding of the ATP analogs ATPγS and the fluorescent analog mant-ATP on the protein stability. Such analogs are used to mimic functional states of the ATPase but undergo different interactions with the binding site that are not well characterized. We propose a competition-based assay for nucleotide binding using CD-spectroscopy to deduce the occupancy of the nucleotide-binding site by non-fluorescent nucleotides. Alternatively, the change of intrinsic fluorescence of mant-ATP upon binding to the ATPase is exploited in these assays. Finally, we show how the simultaneous measurement of protein CD and nucleotide fluorescence in thermal denaturation experiments may help to determine the stability of several functional conformational states of CopB.

P-489

TRPM8 mRNA and protein expression in rat vas deferens

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The transient receptor potential (TRP) channel TRPM8 is a Ca^{2+}-permeable nonselective cation channel activated by cold and the cooling compounds menthol and icilin. TRPM8 is expressed in sensory neurons, where its functions lie in temperature detection. TRPM8 is also expressed in various internal tissues, e.g. vas deferens, where temperature is stable in physiological conditions, suggesting that TRPM8 is involved in other, temperature unrelated processes. RT-PCR analysis showed the presence of *TRPM8* RNA in isolated total RNA from tissue of rat vas deferens. By sampling exclusively smooth muscle cells of the isolated rat vas deference PCR analysis showed no amplification of the aimed cDNA. However we found the shortened amplicon, which could correspond to *TRPM8* splice variant. We performed Western blot analysis that showed the expression of TRPM8 protein in rat vas deference tissues. Our results could suggest the probable exon deletion in transmembrane part of the receptor, resulting in possible expression of electrophysiologically unfunctional TRPM8 receptor in rat vas deferens.

P-490

Computation of Poisson-Nernst-Planck equations describing ion channel on cell membrane

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Keywords: Ion channel, Poisson-Nernst-Planck equations, Chebyshev pseudospectral method, finite size effect.

Ion channels are proteins with a hole down the middle embedded in cell membranes. Membranes form insulating structures and the channels through them allow and control the movement of charged particles, spherical ions, mostly Na+, K+, Ca++, and Cl- Ion channel is extremely important in physiology. Many diseases are related to the malfunction of ion channels, and the drug market involved with ion channel is huge. Though important, the mechanism of ion channel still remains poorly understood nowadays especially about gating and ion selectivity. One of the reasons is that it is lack of a good mathematical model. The traditional model for ion channel is Poisson-Nernst-Planck (PNP) equations. Though PNP equation can produce some results and agree well with experiments, this model still can not explain the mechanisms mentioned above. Recently, a modified PNP model considering finite size effect of ions has been derived from energetic variational analysis. This new model may shed some lights on the explanation of gating and selectivity mechanisms. Though progress is gained in mathematical modeling, few numerical works were reported. This is because that PNP equations are not that simple to solve and the complexity of channel geometry makes the problem even harder. This paper applies Chebyshev pseudospectral method to solve PNP equation under a 2D axis-symmetric configuration with and without considering size effect (a Leonard Jones potential nonlocal integral term). Since we are only interested in steady state most of time, pseudo-time-dependence is introduced to Poisson equation and turns the original elliptic-parabolic problem into a pure parabolic one, which saves the cost of solving Poisson equation in each time step. The results showing the steady-state distribution of electric potential, ionic concentrations are obtained efficiently. Channel current, a summation of drift and diffusive currents, can be further computed from the flux of ionic concentrations. The influence of finite size effect will be also addressed.

O-491

Effect of cholesterol and cytoskeleton on K_V10.1 membrane distribution

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The potassium channel K_V10.1 is expressed nearly exclusively in the central nervous system. Besides its function as an ion channel, K_V10.1 has also been associated with non-canonical signaling functions. Various membrane proteins associated with cholesterol-sphingolipids enriched microdomains are involved in signaling pathways. In this work we studied the membrane distribution of K_V10.1 in highly purified brain-tissue plasma membranes as a function of cholesterol content versus

cytoskeletal proteins. The results show that one fraction of KV10.1 associates to cholesterol-rich domains or Detergent Resistant Membranes (DRM) and another fraction to non-DRM domains. The KV10.1 fraction inserted in DRM is dependent on cholesterol as well as on cytoskeleton proteins. Depletion of cholesterol leads to a doubling of K_V10.1 current density. We suggest that K_V10.1 coexists in two different populations: one where the transmembrane domain fits cholesterol enriched membranes and another able to fit into a less packed lipid bilayer. The importance of this distribution on signaling processes needs to be further investigated.

P-492
Effect of charge fluctuations on the ionic escape rate from a single-site ion channel
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We analyse the permeation of an open, single-site, ion channel by use of Brownian Dynamics (BD) simulations at different concentrations. We show for the first time by modelling that the ionic escape rate increases significantly with concentration due to electrostatic amplification of charge fluctuations at the channel mouth.
We use the reduced model of an axis-symmetric water-filled channel whose protein wall has a single charged site. The channel length, radius and fixed charge are selected to match experimental data for Gramicidin A. The ion current, occupancy and escape rate are simulated by the 1D self-consistent BD technique with account taken of the electrostatic ion-ion interaction. The bath with non-zero ion concentration on one side of the channel is modelled via the Smoluchowski arrival rate.
It is shown that: a) The occupancy saturates with Michaelis-Menten kinetics. b) The escape rate starts from the Kramers value at small concentrations and then increases with concentration due to the electrostatic amplification of charge fluctuations. The resulting dynamics of the current can be described by modified reaction rate theory accounting for ionic escape over the fluctuating barrier [1].

[1] D. G. Luchinsky et al, J. Stat. Mech. (2009), 01010

P-493
Functional reconstitution of membrane proteins by isothermal titration calorimetry
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Many membrane-protein functions are amenable to biophysical and biochemical investigation only after the protein of interest has been reconstituted from a detergent-solubilised state into artificial lipid bilayers. Unfortunately, functional reconstitution has remained one of the main bottlenecks in the handling of numerous membrane proteins. In particular, gauging the success of reconstitution experiments has thus far been limited to trial-and-error approaches. To address this problem, we have established high-sensitivity isothermal titration calorimetry (ITC) as a powerful method for monitoring the reconstitution of membrane proteins into liposomes. ITC has previously been employed for characterising liposome solubilisation and reconstitution in the absence of protein. Here we show that ITC is also excellently suited for tracking the complex process of membrane-protein reconstitution in a non-invasive and fully automated manner. The approach is exemplified for the prokaryotic potassium channel KcsA, which we first purified in detergent micelles and then reconstituted into stable proteoliposomes at very high protein densities. Electrophysiological experiments performed in planar lipid membranes confirmed that KcsA regained its functional activity upon ITC-guided reconstitution.

O-494
Gating currents of low-voltage-activated T-type calcium channels family
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T-type calcium channels are distinguished by relatively low voltage threshold for an activation and steep voltage dependence of activation and inactivation kinetics just above the activation threshold. Kinetics and voltage dependence of macroscopic inward calcium current through Ca_V3 channels was described in a detail. In contrast, very little information is available on gating current of these channels. Therefore we compared gating currents measured from all three $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ channels.
Voltage dependencies of charge movement differ dramatically from those for macroscopic current. First, their slope factors are several-fold bigger that slope factors of macroscopic current activation. Second, activation mid-point for $Ca_V3.3$ channels on-gating is shifted to more positive membrane potentials by about 20 mV compare to $Ca_V3.1$ and $Ca_V3.2$ channels, whose activation mid-points are similar. The same is truth for off-gating voltage dependences. Kinetics of both on- and off-gating is remarkably faster for $Ca_V3.1$ and $Ca_V3.2$ channels compare to $Ca_V3.3$ channels. Further, more charge is moved per unit of macroscopic current amplitude in $Ca_V3.3$ channels compare to $Ca_V3.1$ and $Ca_V3.2$ channels.

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O-495
Differential modulation of inactivated states by hydrophilic and hydrophobic sodium channel blockers
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The local anaesthetic lidocaine (LID) is generally believed to reach its binding site in the intracellular vestibule of the voltage-gated sodium channel via the cell membrane. QX222

(QX) is a permanently charged, quaternary amine analogue of LID, that can access this binding site via a hydrophilic route across the channel protein. The mutation I1575E of the adult rat muscle-type sodium channel (rNa$_V$1.4) opens such a hydrophilic pathway.
When bound to the internal vestibule, LID stabilizes both fast and slow inactivated states. We wondered whether QX, once bound to the internal vestibule, exerts a similar modulatory action on inactivated states as LID. The construct I1575E was expressed in tsA201 cells and studied by means of the patch-clamp technique.
When applied from the extracellular side 500 μM QX stabilized the slow but not the fast inactivated state in I1575E. When applied internally, QX entered the channel, but stabilization of inactivated states could not be observed.
These results suggest that binding site for use-dependent block is in the inner vestibule of the channel, fast inactivation is modulated only by the hydrophobic form of LID, and the binding site for modulation of slow inactivation by QX is only accessible from the extracellular side of the channel.

P-496
Membrane composition effects on pore-forming ability of two cholesterol-dependent cytolysins
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Perfringolysin O (PFO) and Listeriolysin O (LLO) are two Cholesterol-Dependent Cytolysins (CDCs) produced by Gram-positive bacteria. Their main task is to facilitate the infection disrupting plasma or endosomal membranes. Even if CDCs have been well studied, their pore-forming mechanism is not clear yet. Two models are proposed: pre-pore to pore transition and arc-shaped pore formation.
Based on this, we investigated the electrophysiological properties and structures of the two toxins pores in different membrane compositions (i.e. phospholipid acyl chains and cholesterol concentration) using Planar Lipid Membrane (PLM) technique and Atomic Force Microscopy (AFM). Our analysis identified two distinct pore-forming abilities. On POPC-CHO 50% membrane, both proteins form well defined pores with conductance values mainly around 10-15 nS, visible by AFM as complete ring inserted into the membrane of about 30 nm diameter. On the other hand on DOPC-CHO 20% membrane, PFO and LLO form pores with heterogeneous conductance (from pS to nS) but in most of the cases, pores are difficult to be sorted out both by PLM and AFM analyses.
Collectively, our results support the idea that prevalence of pre-pore or arc-shaped oligomeric intermediates are promoted by different membrane lipid compositions.

P-497
Concerted or sequential conformational transitions in Kv7.1 channel activation gating?
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The assembly of Kv7.1 with KCNE1 produces the I_{KS} K^+ current that is crucial for the repolarization of the cardiac action potential. Mutations in these subunit genes produce the long QT syndrome, a life-threatening ventricular arrhythmia. Despite numerous studies performed on *Shaker*-like Kv channels, very little is known about the mechanisms underlying Kv7.1 channel activation. Do the voltage-induced conformational changes in the Kv7.1 tetrameric complex gives rise to cooperativity in channel function? If so, do they involve concerted or sequential conformational transitions in subunit interactions along Kv7.1 channel activation? To determine the nature of subunit interaction, we introduced one voltage sensor domain (VSD) mutation, R231W, into one, two, three, or four subunits by using a concatenated tetrameric channel construct. When present in all 4 VSDs, the S4 R231W mutation produces instantaneous and voltage-independent K^+ currents by "locking" the channel in the open state. When present in only one VSD, the R231W mutation does not produce an instantaneous current but causes a left shift in the channel activation curve. The other combinations of mutated VSDs suggest a sequential conformational transition in Kv7.1 channel gating.

P-498
Different chemical properties predict resting and inactivated affinity of sodium channel inhibitors
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Affinity of inhibitor compounds to resting and inactivated conformations of sodium channels (Kr and Ki) were investigated: Their correlations with chemical descriptors of inhibitors were studied, in order to deduce the nature of chemical interactions involved in binding to both conformations. Two separate approaches were used: (1) We performed a literature search, calculated Kr and Ki values, and created a database of altogether 204 Kr-Ki pairs obtained from 73 publications. (2) We carried out a comparative electrophysiological study of 35 drugs using rNav1.2 expressing HEK 293 cells and the QPatch automatic patch-clamp instrument.
We observed that lipophilicity (quantified by the logarithm of the calculated water-octanol partition coefficient, logP) is important in determining both Kr and Ki, but had a greater effect on Ki. Distribution coefficients (logD) discriminated better between Kr and Ki than partition coefficients (logP). The ratio of positively charged/neutral forms (quantified by the acidic dissociation constant, pKa) was a significant determinant of resting affinity: predominantly charged compounds tended to be more potent against resting channels, while neutral compounds tended to be more state-dependent. Aromaticity was more important for inactivated state affinity.

P-499

The Na+/H+ exchanger NHE7, biochemical features, intracellular localization and biological function

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Keywords: Acidification of intracellular compartments, Na^+/H^+ exchanger, somatic cell genetics, kinetic measurements, neurological diseases

The acidification of intracellular compartments is critical for a wide range of cellular processes. A recent candidate for pH regulation within early endosomes and TGN is the highly conserved intracellular Na^+/H^+ exchanger 7 isoform (NHE7), whose mutation leads to neurological syndromes in human patients. However, due to its intracellular localization, NHE7 biochemical features are still poorly characterized and its biological function remains elusive.
We have developed somatic cell genetic techniques that enable the selection of variant cells able to resist H^+ killing through plasma membrane expression of H^+ extruders. This enabled us to obtain stable cell lines with forced plasma membrane expression of NHE7. We used them to measure its functional and pharmacological parameters with high accuracy, using fast transport kinetics. To summarize, this exchanger displays unique features within the NHE family, especially with respect to its affinity for its substrates, lithium, sodium and protons and for its guanidine-derived inhibitors. Taken together with our results on the subcellular localization of the native NHE7, these unique biochemical features provide new insights on the biological function and pathological implications of this intracellular Na^+/H^+ exchanger.

P-500

Analysis of the collective behaviour of ion channels

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A novel approach to the analysis of the ion current recordings is proposed. The main goal of the standard patch clamp technique is to measure single channel activity (however the whole cell configuration is also used in various researches). In the presented study the ion channels time series recordings were several (up to four) ion channels were present are analysed and the collective behaviour of ion channels is investigated. The time ion current time series are converted into dwelltime series and the channel activity is analysed. The hypothesis of collective ion channels behaviour is verified and the influence of organolead compounds (Met3PbCl) on collective ion channel activity is measured. The analysis is performed on the SV cation channels of vacuolar membrane of Beta vulgaris.
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P-501

On motor and electrical oscillations in urinary tract: computer evaluation

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Introduction: Motor/electrical activity of urogenital tract is fundamental for patho-physiology (incontinence, reflux, etc. can lead to renal hypertonia).
Method: Parameters: *Motor patterns* (guinea-pig) – frequency/F, amplitudes/A (% init. length, isot. & intracell. rec.) of *spontaneous phasic/SPC & tonic/STC contractions,* also ***electrical*** *spikes/S, bursts/B, burst plateaus/BP* (Neu et al. Biophys.J. 125/6a/Jan2008; Eur.Biophys.J. 36/S1,S150/2007 & 34/6,765/2005; J.Physiol.Sci. 59/S1,249/2009; Faseb J. 19/4,139.4/2005).
Results: SPC-pyelon F:5.4±6.1/min, A:4.7±2.7%, SPC-ureter F:1.4±0.7, A:5.3±3.2, SPC-detrusor F:3.8±0.9, A:18.7±5.3, STC-trigone F:0.3±0.1, A:74.6±14.8; ureter-S: F:18.0±4.8/min, A:42.8±5.5mV, -BP: F:7.2±4.8, A:47.5±3.0, -BP-duration 1.3±0.9s; detrusor-S: F:15.0±7.8, A:47.6±7.3; -BP: F:1.1±0.9, A:52.3±9.0, -BP-duration 9.9±3.0s (n=327, $p<0.01$). Stretch (3-80mN), K-/Ca-influence induced specific changes in motor/electrical parameters. Special computer programme reflects exactly biophysical parameters.
Conclusion: Acc. to earlier/recent results mechano-sensitive Ca^{++}-activated K^+-channels participate in electrical oscillations of detrusor/ureteral myocytes. Further experiments/evaluations incl. computer analysis are necessary to prove correlation with motor patterns.

O-502

Molecular rearrangements during slow inactivation of the *Shaker*-IR potassium channel

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Crosstalk between the activation and slow inactivation gates in *Shaker* potassium channels is now well-established. The activation gate perceives the conformation of the inactivation gate (Panyi and Deutsch, 2006, 2007). Closure of the inactivation gate speeds opening and slows closing of the activation gate, i.e., stabilizing the gate in the open configuration. If this coupling involves movement of the S6 transmembrane segment, then we predict state-dependent changes in accessibility of residues lining the channel cavity. We engineered cysteines, one at a time, at positions 470,

471, 472, 473, and 474 in a T449A *Shaker*-IR background and determined modification rates for the cysteine modifying reagents, MTSET and MTSEA, in the open, closed, and inactivated state of the channel. Neither reagent, applied from the intracellular side, modifies cysteines at 470-474 in the closed state. Both 470C and 474C are rapidly modified in the open state and at approximately one-tenth this rate in the inactivated state. In contrast, 471C is not modified in the open state but can be modified by MTSEA but not MTSET in the inactivated state. Residue 472C cannot be modified in any of the three states. Mutant 473C did not express current. Our findings are consistent with a rotation of S6 in the inactivated state, which increases the accessibility of residue 471 while simultaneously decreasing accessibility of residues 470 and 474. Any model of C-type inactivation in the *Shaker* Kv channel must conform to these experimental observations.
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P-503
Mapping of an I_{KS} channel opener reveals interactions between KCNE1 and the Kv7.1 voltage sensor
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In the heart, assembly of Kv7.1 with KCNE1 generates the repolarizing K^+ current I_{KS}. We and others recently suggested a strategic location of KCNE1 wedged between two adjacent Kv7.1 voltage sensing domains (VSD) and nearby helix S6 of another Kv7.1 subunit. Here we show that the I_{KS} channel opener, diisothiocyanostilbene-2',2'-disulfonic acid (DIDS) acts on I_{KS} to convert the time- and voltage-dependent channels into nearly voltage- and time-independent currents. The two isothiocyanate functionalities are crucial for the potent activating effect of DIDS on $I_{KS,}$ since SITS that has only one of these groups and DNDS, which lacks isothiocyanate groups do not activate I_{KS} currents. DIDS does not affect Kv7.2 and has a weaker effect when KCNE1 is co-expressed with a chimeric Kv7.1 bearing a Kv7.2 VSD paddle. Mutagenesis data suggest that DIDS may activate I_{KS} by interacting to an external pocket, formed at the interface of the superficial boundary of the KCNE1 transmembrane segment and the VSD of Kv7.1. DIDS interaction at the Kv7.1 VSD-KCNE1 interface reveals the importance of two lysine residues, K41 in KCNE1 and K218 in Kv7.1 S3-S4 linker and underscores the proximity of KCNE1 to the VSD of Kv7.1.

P-504
An in-silico approach to primaquine binding to Trp756 in the external vestibule of sodium channel Nav 1.4
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The aim of our computed study was to examine the possible binding site of primaquine (PQ) using a combined homology protein modeling, automated docking and experimental approach. The target models of wild-type and mutant-types of the voltage-dependent sodium channel in rat skeletal muscle (rNa_v 1.4) were based on previous work by Tikhonov and Zhorov. Docking was carried out on the P-loop into the structure model of rNa_v 1.4 channel, in the open state configuration, to identify those amino acidic residues important for primaquine binding. The three-dimensional models of the P-loop segment of wild types and mutant types (W402. W756C, W1239C and W1531A at the outer tryptophan-rich lip, as well as D400C, E755C, K1237C and A1529C of the DEKA motif) helped us to identify residues playing a key role in aminoquinoline binding. In good agreement with experimental results, a 1000-fold inhibition loss was observed, tryptophan 756 is crucial for the reversible blocking effects of PQ. As a result, W756C abolished the blocking effect of primaquine in voltage-clamp assays.

P-505
Hydrogen bond formation accelerates channel opening of the bacterial mechanosensitive channel MscL
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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer (TM1, TM2) α-helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. To address this question, we performed molecular dynamics (MD) simulations for the opening of MscL embedded in the lipid bilayer. In the closed state of MscL, neighboring TM1 inner helices are crossed each other near the cytoplasmic side and Leu19 and Val23 in the constricted part form a stable hydrophobic environment called gate. Upon membrane stretch, Phe78 in TM2 outer helices was dragged by lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. During opening, TM1inner helices were also dragged and tilted, accompanied by the outward sliding of the crossings. This led to a slight expansion of the gate associated with an exposure of oxygen atoms of the backbone to the inner surface of the gate. This allows water penetration in the gate and formation of hydrogen bonds between water and the exposed oxygen, which in turn weakened the hydrophobic interaction at the crossings, causing a further opening of the gate and water permeation.

O-506
Molecular characterization of BK_{Ca} channel in cardiac mitochondria
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Mitochondrial BK_{Ca}, mitoBK_{Ca} has been proposed to be cardioprotective and formed by proteins of $\sim$50 to $\sim$125

kDa. Thus, we investigated the molecular characteristics of this channel in isolated mitochondria from murine heart. Labeling of adult mouse cardiomyocytes with plasmalemma BK_{Ca} antibodies, mitotracker, and wheat germ agglutinin yielded remarkable mitochondrial but not plasma membrane localization. Nanoscale fluorescence microscopy (Stimulated Emission Depletion) revealed 7 to 15 of ~40-50 nm BK_{Ca} clusters per mitochondria. Further, Western blot analysis of purified mitochondria showed the presence of a full length ~125 kDa protein. Systematic RT-PCR exon scanning of isolated cardiomyocyte mRNAs were consistent with a full length ~125 kDa alpha-subunit protein and revealed the expression of three splice inserts. Insertless-BK_{Ca} robustly localized to the plasma membrane of CHO cells but when a C-terminal splice insert was present BK_{Ca} was readily targeted to the mitochondria (protein proximity index was ~1.0 indicating 100% colocalization). Hence, cardiac mitoBK_{Ca} is composed by full-length BK_{Ca} protein but with splice inserts which may facilitate its targeting to mitochondria. Supported by NIH and AHA.

P-507

Modulation of slowly activating (SV) channels in red beet (*Beta vulgaris* L.) taproots by organo-lead and –tin.

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Patch-clamp technique was used to examine effect of trimethyl-lead and -tin on the SV channel activity in the red beet (*Beta vulgaris L.*) taproot vacuoles. It was found that the addition of both investigated compounds to the bath solution inhibit, in a concentration-dependent manner, SV currents. When single channel properties were analyzed, only little channel activity can be recorded in the presence of 100 μM of organometal. Compounds investigated decreased significantly (by about one order of magnitude) the open probability of single channels. The recordings of single channel activity obtained in the presence and in the absence of organometal showed that compounds only slightly (by ca. 10%) decreased the unitary conductance of single channels. It was also found that organometal diminished significantly the number of SV channel openings, whereas it did not change the opening times of the channels. Taken together, these results suggest that organometal binding site is located outside the channel's selectivity filter and that the inhibitory effect of both compounds investigated on SV channel activity probably results from organometal-induced disorder in compatibility between membrane lipids and membrane proteins.

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P-508

Electrophysiological investigation of the hVDAC1 ion channel in pore-spanning membranes

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The human Voltage-Dependent Anion Channel 1 (hVDAC1) plays an important role in cell life and apoptosis since it is the main porin of the outer mitochondrial membrane. As hVDAC1 is believed to play a pivotal role in apoptosis-related diseases such as stroke, Alzheimer, Parkinson and cancer, the alterations of its electrophysiological properties under different conditions are of great value.

To perform different investigations, refolded hVDAC1 is reconstituted in artificial membranes which typically consist of DPhPC with a cholesterol fraction of 10–30%. They are prepared *via* the Müller-Rudin-technique on a functionalized porous alumina substrate containing pores with a diameter of 60 nm. The quality of these so-called nano-Black-Lipid-Membranes (nano-BLMs) is verified via electrochemical impedance spectroscopy (EIS), hVDAC1 is reconstituted and single channel recordings are made. Membranes are also prepared by spreading proteoliposomes on hydrophobized porous silicon nitride with pores of 1 μm diameter.

Information about altered gating-characteristics and related conductivities is gained by application of holding potentials up to ±100 mV and evaluation of the resulting currents.

Acknowledgment
The hVDAC1 was a kind gift of Prof. C. Griesinger, MPIBPC, Göttingen.

P-509

MD simulations reveal mutation induced reorientation of binding residues in the hERG K+ channel

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Pharmacological inhibition of cardiac hERG K^+ channels is associated with increased risk of arrhythmias. Many drugs bind directly to the channel, thereby blocking ion conduction. Ala-scanning mutagenesis identified residues important for drug block. Two aromatic residues Y652 and F656 were found to be crucial for block of most compounds. Surprisingly, some cavity blocking drugs are only weakly affected by mutation Y652A. In this study we provide a structural interpretation for this observation. MD simulations on the Y652A mutant suggest side chain rearrangements of F656 located one helical turn below Y652. Loss of π-π stacking induces reorientation of F656 from a cavity facing to a cavity lining conformation, thereby substantially altering the shape of the binding site. Docking studies reveal that due to their rigid shape and compactness Y652 insensitive drugs can still favorably interact with the reoriented F656 aryl groups, while molecules with more extended geometries cannot.

P-510

Clues to understanding the voltage regulation of TRPA1

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The ankyrin transient receptor potential channel TRPA1 is a transmembrane protein that plays a key role in the transduction of noxious chemical and thermal stimuli in nociceptors. In addition to chemical activation, TRPA1 can be activated by highly depolarizing voltages but the molecular basis of this regulation is unclear. The transmembrane part of the tetrameric TRPA1 is structurally related to the voltage-gated K^+ channels in which the conserved charged residues within the fourth transmembrane region (S4) constitute part of a voltage sensor. Compared to these channels, the voltage-dependence of TRPA1 is very weak (apparent number of gating charges $\sim$ 0.4 versus 12 in K^+ channels) and its putative voltage-sensing domain most likely lies outside the S4 because TRPA1 completely lacks positively charged residues in this region. In the present study we used homology modelling and molecular dynamics to create models of the transmembrane part and the proximal cytoplasmic C terminus of TRPA1. In combination with electrophysiological data obtained from whole cell patch-clamp measurements we were able to point out several positively charged residues which mutation strongly alter the voltage sensitivity of TRPA1 channel. These may be candidates for as yet unrecognized voltage sensor.

Photosynthesis

P-511

Action of double stress on Photosystem 2

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The simultaneous effect of photoinhibitory illumination and toxic action of heavy metals ions (Cd^{2+} and Co^{2+}) on activity of PS2 in vitro measuring by millisecond delayed fluorescence (ms-DF) of chlorophyll A was studied. During action on chloroplasts only of Cd^{2+} (10^{-3}M) the fast component of ms-DF, which originates via radiative recombination of reaction center with the $CaMn_4$-cluster or Y_Z on donor side of PS2, is inhibited stronger than at action of only Co^{2+}. The steady-state level at Cd^{2+} treatment is remain stable, while at Co^{2+} action it is increased. Simultaneous action of Cd^{2+} and photoinhibitory illumination (4000 μmol photons m^{-2} s^{-1}) have shown that fast component of ms-DF was inhibited faster with time than in case of action of Co^{2+} and excess light. Result indicates that damage sites of action Cd^{2+} and Co^{2+} are donor and acceptor side of PS2, accordingly. We assume that binding site of Cd^{2+} is Y_Z or $CaMn_4$-cluster, one of the recombination partners with $P_{680}{}^+$ on the donor side of PS2. Thereby, action of Cd ions on donor side of PS2 leads mainly to development of mechanism of donor-side photoinhibition.

P-512

Field instrument for determination of the photosynthetic capacity of intact photosynthetic bacteria

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A combined pump and probe fluorometer and spectrophotometer with high power laser diodes has been constructed to measure fast induction and relaxation of the bacteriochlorophyll fluorescence yield and light-induced absorption changes in intact cells of photosynthetic bacteria. The construction is the upgraded version of our previous set up[1] with better time resolution (5 μs). The compact design of the mechanics, optics, electronics and data processing makes the device easy to use as outdoor instrument or to integrate into larger measuring systems. The versatility and excellent performance of the apparatus will be demonstrated on different fields: 1) organization and redox state of the photosynthetic apparatus of the whole cells under different growth conditions deduced from fluorescence characteristics including the lag phase, the amplitude and the rise time of the variable fluorescence, 2) electron transfer in the reaction center, cytochrome bc_1 complex and in between obtained from relaxation of the fluorescence and 3) re-reduction kinetics of the oxidized primary donor of the reaction center and energetization and relaxation of the intracytoplasmic membrane tracked by absorption changes at 798 and 525 nm, respectively.

(1) Kocsis et al. Photosynth Res (2010) 105:73–82.

P-514

Role of carotenoid and aggregation in energy dissipation of IsiA from cyanobacteria

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Previous work has established that the Iron Stress Induced protein A (IsiA) synthesized by cyanobacteria under stress conditions, has at least two functions: light harvesting [1] and photoprotection [2]. Under prolonged iron starvation IsiA becomes the main chlorophyll-binding protein in the cell and occurs without a photosystem association. These IsiA aggregates have a strong ability to dissipate light energy and there is evidence of carotenoid participation in the quenching mechanism via downhill energy transfer from chlorophyll to the S1 state of a carotenoid [3]. In the present work we have measured the temperature dependence of the fluorescence of carotenoid depleted mutants (echinenone and/or zeaxanthin) and IsiA monomers in order to investigate the role of carotenoid and aggregation in the quenching process. Pigment analysis confirms the absence of the carotenoid mutated in its biosynthesis but shows that it is mainly replaced. The monomers are lacking two carotenoids, echinenone and one of the two ß-carotenes found previously in IsiA aggregates. Temperature dependent fluorescence shows that quenching properties are affected in the monomers and the mutants lacking zeaxanthin.

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O-515

Photosynthetic water oxidation: from biophysics to solar fuels

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Soon exhausted oil resources and global climate change have stimulated research aiming at production of alternative fuels, ideally driven by solar energy. Production of solar fuels needs to involve the splitting of water into protons, energized electrons and dioxygen. In photosynthetic organisms, solar-energy conversion and catalysis of water splitting (or water oxidation) proceed in an impressive cofactor-protein complex denoted as photosystem II (PSII). The heart of biological water-oxidation is a protein-bound manganese-calcium complex working at technically unmatched efficiency. In an attempt to learn from nature, the natural paragon is intensely studied using advanced biophysical methods. Structural studies by X-ray spectroscopy with synchrotron radiation play a prominent role in this endeavor. Time-resolved methods provide insights in the formation of intermediate states of the reaction cycle. An overview is presented focusing on (i) the efficiency of solar energy usage in PSII, (ii) the interrelation between electron transfer and proton relocations, and (iii) the mechanism of water oxidation. As an outlook, new results on water oxidation by biomimetic manganese and cobalt oxides, which may become a key element in future solar-fuel systems, are presented.

P-516

Energy harvesting in photosynthetic antennas: structural and dynamical characterization of non-equivalent peridinins in different forms of the Peridinin-Chlorophyll-Protein

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The Peridinin-Chlorophyll-Protein (PCP) is a light-harvesting complex (LHC) that works as antenna in the photosynthetic process of dinoflagellates. The protein contains both chlorophylls and carotenoids molecules, the latter being responsible to extend the spectral range of captured light to regions where chlorophylls are transparent. PCP crystal structures [1] reveal that each chlorophyll is surrounded by 3 or 4 molecules of the carotenoid peridinin, located in non-equivalent positions. The different protein environment of the sites might be responsible of a spectral shift of the pigments with the functional role to extend the absorption spectra of the complex and enhance its light harvesting capabilities. High resolution X-ray diffraction data on a reconstructed PCP, the refolded peridinin-chlorophyll a-protein (RFPCP) [2], and on the less common high salt-PCP (HSPCP) opened the way to the mechanistic understanding of peridinin spectral tuning, peridinin chlorophyll energy transfer and photoprotective mechanism [3]. The two PCP forms differ in various features: spectral properties, molar mass, amino acid sequence and, above all, pigment stoichiometry, the peridinin:chlorophyll ratio being 4:1 for the RFPCP and 3:1 for the HSPCP [4]. In the present work we perform classical molecular dynamics simulations of the RFPCP and the HSPCP in explicit water solution. We analyse the structure and dynamics of the proteins and of their pigments to characterize the different peridinin sites in both PCP forms in terms of quantities that can affect the chromophore spectra, such as distorsion, fluctuations and nature of the protein environment. The comparison between the data suggests correspondences between the pigments of the two forms. Quantum and mixed quantum/classical molecular dynamics simulations are also under progress to investigate the effect of the protein environment on the electronic and optical properties of the PCP pigments.

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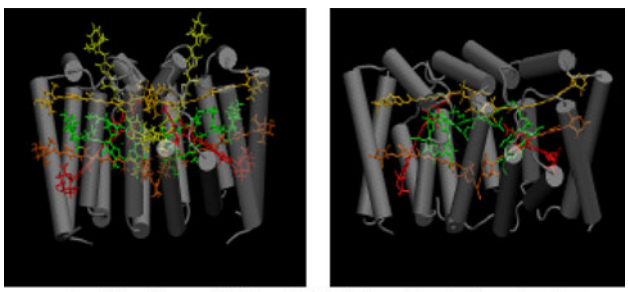

Two different forms of PCP and their cofactors: chlorophyll (green) and peridinin (from yellow to red) molecules
Peridinin:chlorophyll ratio 4:1 for RFPCP (left) and 3:1 for HSPCP (right)

P-518

Porous silicon/photosynthetic reaction center hybrid nanostructure

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Multifunctional self-assembled nanoscale materials based on bio-matter are of special interests because of promising

peculiar applications, like in optoelectronics, biosensors, photovoltaics. Among the existing carrier matrices conductive metal oxides (e.g. indium tin oxide, ITO), carbon nanotubes, graphenes, silicon (Si) are the most frequently used materials because of their unique characteristics such as good conductivity, good optical properties and excellent adhesion to substrates. In our work we combined purified photosynthetic reaction center protein (RC) and porous silicon (PSi) investigating the morphology and optoelectronic properties of the bio-nanocomposite material. FTIR spectroscopy, scanning electron microscopy and energy-dispersive X-ray spectroscopy indicated the binding of the protein to the PSi. Specular reflectance spectra showed a red shift in the characteristic interference peak of the PSi microcavity which was saturated at higher concentration of the protein. The binding was more effective if the functionalization was done by the Si-specific oligopeptide compared to the classical covalent binding via aminopropyl-triethoxysilane (APTES). Excitation by single saturation flashes indicated that the RC still exhibited photochemical turnover after the binding.

P-519

Effects of singlet oxygen elicitors on plant leaves – are these all caused by ROS?

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The role of reactive oxygen species (ROS) in plant stress, both as damaging agent and as potential signal molecule is often assessed in experiments using photosensitized elicitor dyes. For these studies, it is essential to know how efficiently these chemicals generate ROS, whether they are specific ROS sources, as well as their cellular localization and additional side effects. The present study addresses these issues using a variety of dyes known and traditionally applied as singlet oxygen ($^1O_2$) sources. Rose Bengal (RB), Methylene Violet (MVI), Methylene Blue (MB), Neutral Red (NR) and Indigo Carmine (IC) were studied as putative ROS sources in tobacco leaves.

ROS products of photosensitized dyes were measured *in vitro*, using spin trapping EPR spectroscopy. Dye concentrations and irradiation concentration leading to equal absorbed excitation quanta were determined spectrophotometrically. *In vivo* studies were carried out using tobacco leaves infiltrated with water solutions of the putative $^1O_2$ sources. Cellular localizations were identified on the basis of the dyes' fluorescence. RB, NR and MVI reached into mesophyll cells and were used to study the effects of these dyes on photosynthesis. Photochemical yields and quenching processes were compared before and after photosensitization of the elicitor dyes inside the leaf samples.

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O-520

Chlorophyll-chlorophyll charge transfer quenching is the main mechanism of non-photochemical quenching in higher plants

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Non-photochemical quenching (NPQ) in plants protects against photochemical destruction of the photosynthetic apparatus under excess light conditions.While one location of the NPQ process has been shown to be centered on the major light harvesting complex II (LHCII) (Q1 type or qEquenching), an additional quenching center responsible for qI type (identical to Q2 center) quenchinghas been suggested to be located on the minor light-harvesting complexes upon accumulation ofzeaxanthin (Zx), in particularon CP24 and CP29

We have performed femtosecond transient absorption and time-resolved fluorescence measurements of NPQ quenching in intact leaves of higher plants, on isolated light harvesting complexes in the minor (non-aggregated)light harvesting complex CP29 reconstituted with violaxanthin (Vx) or Zx, and in the isolated major LHC II complex in the aggregated state. In all of these situations we find the formation of Chl-Chl charge transfer (CT) states to be the dominant quenching mechanism. The yield of formation of carotenoid cation states and/or carotenoid S_1 state is either extremely low or absent, thus excluding their involvement in NPQ quenching as a major quenching mechanism.

P-521

Fluorescence dynamics of plant light harvesting complexes studied by single molecule spectroscopy

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Single-molecule spectroscopy (SMS) is a powerful technique that allows investigation of fluorescence properties from single fluorescing systems. This technique enabled us to investigate the dynamics of the fluorescence intensity and spectral profiles of single, isolated light harvesting complex (LHC) on timescales of milliseconds to seconds, during continuous laser illumination. We were able to observe how each complex can rapidly switch between different emission states [1,2] and to characterise the intensity and the spectral dynamics of major and minor antenna complexes from plants, in two different environments, mimicking the light harvesting and the light dissipating state, respectively. The results will be discussed with respect to the current models for nonphotochemical quenching (NPQ) mechanisms [3,4,5],

a vital photoprotection mechanism during which the LHCs of plants switch between a state of very efficient light utilisation and one in which excess absorbed excitation energy is harmlessly dissipated as heat.

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P-522

Characterization of the light harvesting antenna system in the diatom *Phaeodactylum tricornutum*

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Phaeodactylum tricornutum is one of the most utilized model organisms in diatom photosynthesis research mainly due to availability of its genome sequence (1). It's photosynthetic antennae are the Fucoxanthin Chlorophyll a/c binding Proteins (Fcps) which share a high degree of homology with Lhcs of higher plants and green algae (2). For detailed investigation of the antenna system of *P.tricornutum*, a transgenic strain expressing recombinant His-tagged FcpA protein was created which simplified the purification of a specific stable trimeric Fcp complex consisting of FcpA and FcpE proteins. Excitation energy coupling between fucoxanthin and chlorophyll *a* was intact and the existence of a chlorophyll *a*/fucoxanthin excitonic dimer was demonstrated (3). We investigated in detail the existence of specific antenna system for PSI and PSII in *P.tricornutum* as in case of higher plants. Our studies indicated that at least the main light harvesting proteins FcpA and FcpE are most probably shared as a common antenna by both PSI and PSII.

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O-523

Effect of hydrophobic mismatch on the light-induced structural changes in bacterial reaction centers

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Light-induced structural changes are reported near the primary electron donor of bacterial reaction centers (BRC) dispersed in detergent micelles and in liposomes from lipids with different fatty acid chain lengths. In this study we present evidence for the correlation between the light-induced increase of the local dielectric constant, determined by the analysis of the electrochromic absorption changes, and the lifetime of the charge-separated state at physiologically relevant temperatures. The increase of the local dielectric constant induced a significant decrease of the oxidation potential of the primary electron donor and a slow proton release, which appears to be the rate limiting step in the overall process. Systematic selection of the head group charges of detergents and lipids, as well as the thickness of the fatty acid chains of the liposome forming lipids can increase the lifetime of the charge-separated state by up to 5 orders of magnitude. Such extensions of the lifetime of the charge-separated state were reported earlier only at cryogenic temperatures and can provide new opportunities to utilize the BRC in energy storage.

P-524

Ontogenesis of photosynthetic bacteria tracked by absorption and fluorescence kinetics

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The development of photosynthetic membrane of *Rhodobacter sphaeroides* was studied by absorption spectroscopy and fast induction of bacteriochlorophyll fluorescence in different phases of the growth, under various growing conditions (oxygen content, light intensity etc.) and in synchronous cell population. The results are: 1) The newly synthesized components of the membranes were imbedded immediately into the proteinous scaffold independently on the age of the cell (no „transient" membranes were observed). 2) Under aerobic conditions, the pigments were bleached and under anaerobic conditions the pigment systems showed greening. The relative variable fluorescence (F_v/F_{max}) had small age-dependent (but not cell-cycle-related) changes. The fluorescence induction kinetics was sensitive marker of the aerobiosis: the F_v/F_{max} ratio dropped from 0.7 to 0.4 and the photochemical rate constant from $5{\cdot}10^4$ s^{-1} to $3{\cdot}10^4$ s^{-1} with an apparent halftime of about 4-5 hours after change from anaerobic to aerobic atmosphere. 3) The electrogenic signal (absorption change at 525 nm) reflected the energetization of the membrane which showed cell-cycle dependent changes. That included periodic production and arrangement of protein-lipid components of the membrane synchronized to the cell division.

P-525

Functional domain size of the plant light-harvesting antenna in native membranes and artificial aggregates

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The degree of energetic connectivity, or functional domain size, was studied in artificial aggregates of the main light-

harvesting complex II (LHCIIb) from spinach or in native thylakoid membranes by picosecond time-resolved fluorescence. The domain size was estimated by monitoring the efficiency of added exogeneous singlet excitation quenchers – phenyl-*p*-benzoquinone (PPQ) and dinitrobenzene (DNB). The fluorescence decay kinetics of the systems under study were registered without quenchers and with quenchers added in a range of concentrations. Stern-Volmer constants, K'_{SV} and K_{SV} - for aggregates (membranes) and detergent-solubilized complexes, respectively, were determined from the concentration dependence of the ratio of the mean fluorescence lifetimes without/with quencher (τ_0, τ). The ratio $K'K_{SV} \bullet \tau_0/\tau'_0$ was suggested as a measure of the functional domain size. Values in the range of 15-30 were found for LHCII macroaggregates and 12-24 for native thylakoid membranes, corresponding to domain sizes of 500-1000 chlorophylls. Although substantial, the determined functional domain size is still orders of magnitude smaller than the number of physically connected pigment-protein complexes; thus our results imply that the physical size of an antenna system beyond these numbers has little or no effect on improving the light-harvesting efficiency.

P-526

Photosynthetic reaction center/carbon nanotube hybrid nanostructures

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The interaction between photosynthetic reaction center proteins (RCs) purified from purple bacterium *Rhodobacter sphaeroides* R-26 and functionalized and non-functionalized (single (SWNT) and multiple (MWNT) walled) carbon nanotubes (CNT-s) has been investigated. Both structural (AFM, TEM and SEM microscopy) and functional (flash photolysis and conductivity) techniques showed that RCs can be bound effectively to different CNTs. Both physical sorption and binding through $-NH_2$ or –COOH groups gave similar results. However, it appeared that by physical sorption some sections of the CNTs were covered by multiple layers of RCs. After the binding the RCs kept their photochemical activity for a long time (at least for three months, even in dried form) and there is a redox interaction between the CNT and RCs. The attachment of RC to CNTs results in an accumulation of positive and negative charges followed by slow reorganization of the protein structure after excitation. In the absence of CNT the secondary quinone activity decays quickly as a function of time after drying the RC onto a glass surface. The special electronic properties of the SWNT/protein complexes open the possibility for several applications, e.g. in microelectronics, analytics or energy conversion and storage.

O-527

Relaxation of bacteriochlorophyll fluorescence in intact cells of photosynthetic bacteria

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The decay of the high fluorescence state generated by actinic illumination of different durations was measured in whole cells of various strains and mutants of photosynthetic purple bacteria. Although similar method is used in higher plants, its application in photosynthetic bacteria is novel and highly challanging. The available data are restricted and only the re-oxidation of the reduced primary quinone ($Q_A^- \rightarrow Q_A$) is usually blamed for the decay kinetics. Here, we will analyse the complexity of the kinetics over a very broad time range (from 5 μs to 5 s) and show that the dark relaxation of the bacteriochlorophyll fluorescence reflects the overlap of several processes attributed to the intra- and interproteinous electron transfer processes of the reaction center (RC) and cytochrome bc_1 complex of the bacterium. In the shorter (< 1 ms) time scale, the dominating effect is the re-reduction of the oxidized primary donor ($P^+ \rightarrow P$) that is followed by the re-oxidation of the acceptor complex of the RC by the cytochrome bc_1 complex. As the life times and amplitudes of the components depend on the physiological state of the photosynthetic apparatus, the relaxation of the fluorescence can be used to monitor the photosynthetic capacity of the photosynthetic bacteria *in vivo*.

P-528

Anisotropic circular dichroism signatures of oriented thylakoid membranes and lamellar aggregates of LHCII

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Circular dichroism (CD) spectroscopy is an indispensable tool to probe molecular architecture. At the molecular level chirality results in intrinsic CD, pigment-pigment interactions in protein complexes give rise to excitonic CD, whereas "psi-type" CD originates from large densely packed chiral aggregates. It has been well established that the anisotropic CD (ACD), measured on samples with defined orientation, carries specific information on the architecture of molecules. However, ACD can easily be distorted by linear dichroism of the sample or instrumental imperfections – which might be the reason why it is rarely studied in photosynthesis research. Here we present ACD spectra of isolated intact and washed, unstacked thylakoid membranes, photosystem II membranes (BBY), and tightly-stacked lamellar macroaggregates of the main light-harvesting complex II (LHCII). We show that the ACD spectra of face- and edge-aligned stacked thylakoid membranes and LHCII lamellae exhibit profound differences in their psi-type CD bands. Marked differences are also seen in the excitonic CD of BBY and washed thylakoid membranes. Thus ACD provides an additional dimension to the

analysis of complex biological structures as compared to isotropic CD.

P-529

Conformation changes after primary charge separation in photosynthetic reaction centers

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Light induced conformation changes of quinone depleted photosynthetic reaction centers (RCs) purified from the carotenoid-less *Rhodobacter sphaeroides* R-26 were investigated by transient absorption (TA) and grating (TG) methods. Surprisingly, the decay of the TA signal measured at 860 nm was divided into a 15 ns and a 40 μs components. The latter coincides with the life time of the TG signal, which was assigned earlier [Nagy et al. (2008) FEBS Lett. 582, 3657-3662] to spectrally silent conformational changes. The nature of the 40 μs phase was investigated further. Although, the probability of chlorophyll triplet formation under our measuring conditions was small, possible contribution of the triplet states was also studied. The presence of carotenoid in the wild type RCs eliminated the 40 μs component indicating the role of carotenoid in the energy transfer within the RCs. There was no significant effect of the molecular oxygen on the TA. This fact may be explained if the chlorophyll triplets inside the protein have reduced accessibility to molecular oxygen. A differential effect of osmotic potential and viscosity on conformation changes accompanying the primary charge separation was measured by the effect of ficoll, glucose and glycerol as compared the TA to the TG signals.

O-530

Variable chlorophyll fluorescence: in part a yield change due to light-induced conformational change

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On a dark-to-light transition the chlorophyll fluorescence rises from a minimum intensity (F_0) to a maximum intensity (F_M). Conventionally, this rise is interpreted to arise from the reduction of the primary quinone acceptor, Q_A, of photosystem II - although this cannot explain all presently available observations. In untreated leaves, at room temperature, the fluorescence rise follows the reduction of the electron transport chain (ETC). Once induced, ~30-40 % of the variable fluorescence intensity relaxes within 100 ms in darkness and can be re-induced within 3 ms as long as the ETC remains reduced. Analyzing the fluorescence relaxation kinetics, +/- DCMU, ~30% of the amplitude cannot be explained by Q_A^- re-oxidation. Special properties of this phase determined on DCMU-inhibited samples: at cryogenic temperatures (below -40°C), where the Q_A^-/S_2 recombination is blocked, it still relaxes and it exhibits a strong temperature dependence with an apparent $E_a \approx 12$ kJ/mol, whereas the reduction of Q_A is nearly temperature insensitive. A fluorescence yield change, driven by light-induced conformational change in the reaction center complex, can explain all these observations.

P-531

Tuning function in bacterial light-harvesting complexes

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Purple photosynthetic bacteria are able to synthesize a variety of different light-harvesting complexes, sometimes referred to as LH2, LH3 and LH4. Here we have investigated the structural origins of these different forms and the manner in which the sequence tunes the absorption spectrum of the light-harvesting system. We then consider the functional consequences of this tuning for the organization of the light harvesting system and on the ecology of the organisms. Specifically by spectroscopic techniques, in particular circular dichroism and resonance Raman spectroscopy, we have been able to obtain information on the organization of the bacteriochlorophyll binding sites in the unusual LH4 of *Roseobacter denitrificans*. This provides a picture of how different peripheral light-harvesting complexes are able to modulate the absorption spectrum. The structure and organization of this complex is the put in the context of the the recently published variability of the light-harvesting complexes. In particular the observation of their ability to form mixed complexes containing different polypeptides. We examine quantitatively the possible reasons for maintaining such variability by considering the transport properties of membranes containing either pure or mixed complexes and show that mixed complexes can permit light-harvesting to continue during adaptation. We then consider the different constraints that may be behind this type of adaptation in different bacteria and the conditions under which different types of antenna system might be optimal Finally we integrate this into the evolutionary context of adaptation to variable light intensity and the ecological niches where such organisms are found.

P-532

Interaction between photosynthetic reaction centers and ITO

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Photosynthetic reaction center proteins (RC) purified from *Rhodobacter sphaeroides* purple bacterium were deposited on the surface of indium-tin-oxide (ITO), a transparent conductive

oxide, and the photochemical/-physical properties of the composite was investigated. The kinetics of the light induced absorption change indicated that the RC was still active in the composite and there was an interaction between the protein cofactors and the ITO. The electrochromic response of the bacteriopheophytine absorption at 771 nm showed an increased electric field perturbation around this chromophore on the surface of ITO compared to the one measured in solution. This absorption change is associated with the charge-compensating relaxation events inside the protein. Similar life time, but smaller magnitude of this absorption change was measured on the surface of borosilicate glass. The light induced change in the conductivity of the composite as a function of the concentration showed the typical sigmoid saturation characteristics unlike if the chlorophyll was layered on the ITO which compound is photochemically inactive. In this later case the light induced change in the conductivity was oppositely proportional to the chlorophyll concentration due to the thermal dissipation of the excitation energy.

P-533

The supramolecular organization of photosystem II in vivo studied by circular dichroism spectroscopy

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The light reactions of photosynthesis in higher plants take place in granal chloroplast thylakoid membranes, which contain chirally organized macrodomains composed of photosystem II (PSII) supercomplexes associated with light harvesting antenna complexes (LHCIIs). The physiological relevance of this hierarchic organization, which often manifest itself in semicrystalline assemblies, has not been elucidated but the diversity of the supramolecular structures and their reorganizations under different conditions indicates its regulatory role. The present work focuses on the structural and functional roles of different components of LHCII-PSII supercomplexes. We used various growth conditions, influencing the protein composition, and different Arabidopsis mutants (KoCP24, KoCP26, KoPsbW, KoPsbX, Dgd1), with altered organization of the membranes, and measured their circular dichroism (CD) spectra as well as their chlorophyll fluorescence kinetics to characterize the chiral macro-organization of the chromophores and the functional parameters of the membranes, respectively. We show that the formation of chiral macrodomains require the presence of supercomplexes. Our data also reveal specific functions of some of the protein or lipid compounds in the light adaptation processes of plants.

O-534

Excitation energy transfer and non-photochemical quenching in photosynthesis

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The success of photosynthesis relies on two ultrafast processes: excitation energy transfer in the light-harvesting antenna followed by charge separation in the reaction center. LHCII, the peripheral light-harvesting complex of Photosystem II, plays a major role. At the same time, the same light-harvesting system can be 'switched' into a quenching state, which effectively protects the reaction center of Photosystem II from over-excitation and photodamage. In this talk I will demonstrate how LHCII collects and transfers excitation energy. Using single molecule spectroscopy we have discovered how LHCII can switch between this light-harvesting state, a quenched state and a red-shifted state. We show that the switching properties between the light-harvesting state and the quenched state depend strongly on the environmental conditions, where the quenched state is favoured under 'NPQ-like' conditions. It is argued that this is the mechanism of non-photochemical quenching in plants.

P-535

Photobiology in the soil: arrested chlorophyll biosynthesis in pea epicotyl sections

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The key regulatory step of chlorophyll (Chl) biosynthesis is the NADPH:protochlorophyllide oxidoreductase (POR) catalyzed reduction of protochlorophyllide (Pchlide)which is light activated in angiosperms. This process is usually described on artificially dark-grown plants.

In this work, we studied epicotyl segments developed under the soil surface, which were dissected from pea plants grown under natural light conditions. Using 77 K fluorescence spectroscopy, pigment analyses, electron microscopy and fluorescence microscopy, we found that upper segments showed transitional developmental stages, i.e. Chl appeared besides Pchl(ide) and etio-chloroplasts were typical. In regions under 5 cm depth, however, the characteristics of the segments were similar to those of plants germinated artificially in complete darkness, i.e. only Pchl(ide) and etioplasts were present.

The results of this work prove that these latter symptoms may occur in shaded tissues of fully developed, photosynthetically active plants grown under natural conditions.

Electron and proton transfer, bioenergetics

O-537
Computational Bioenergetics: From pure electron transfer in cytochromes to chemistry in catalases

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In this overview talk it will be shown how atomistic computations can complement experimental measurements in our quest to understand biological electron and proton transfer reactions. At first the molecular simulation methods for calculation of important electron transfer parameter such as reorganization free energy, electronic coupling matrix elements and reduction potentials will be explained. Then three applications will be discussed where such computations help interpret experimental data on a molecular level. The first example concerns electron tunneling between heme a and heme a3 in the proton pump cytochrome c oxidase. This reaction is very fast, occurring on the nano-second time scale, and it is unclear if this is due to an unusually low reorganization free energy or due to high electronic coupling. Carrying out large-scale all-atom molecular dynamics simulation of oxidase embedded in a membrane, we do not find evidence for unusually small values of reorganization energy as proposed previously, implying that the nanosecond tunneling rate between heme a and a3 is supported by very efficient electronic coupling. The second example is on electron transport in a deca-heme 'wire'-like protein, used by certain anaerobic bacteria to transport electrons from the inside of the cell to extracellular substrates. The crystal structure of such a protein has been solved recently for the first time. However, it is unclear if and in which direction the wire structure supports electron transport. Here we present results of heme reduction potential calculations that help us reveal the possible electron flow in this protein. In a third example we explain how quantum mechanical/molecular mechanical methods (QM/MM) recently helped us understand why the catalase from *H. Pylori* is prone to undergo an undesired protein radical migration reaction during catalysis.

P-539
Proton pumping activity of purple and brown membranes regenerated with retinal analogues

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The retinal protein bacteriorhodopsin (BR) acts as a light-driven proton pump in the purple membrane (PM) of *Halobacterium salinarium* (*H.s.*). The aim of these studies was to clarify whether the specific crystalline structure of protein and protein-substrate interactions are significant for H^+ transfer into the aqueous bulk phase. Two membrane systems were prepared: purple membranes (BR arranged in a two-dimensional hexagonal lattice) and brown membranes (BR not arranged in a crystalline lattice) were regenerated with 14-Fuororetinals. Light-induced proton release and reuptake as well as surface potential changes inherent in the regenerated systems reaction cycles were measured. Signals of optical pH indicators residing in the aqueous bulk phase were compared with signals of pH indicator covalently linked to the extracellular surface of proteins and with surface potential changes detected by the potentiometric probe. The energies of activation of proton transfer have been calculated. Experimental results and thermodynamic parameters (energies of activation) suggest the different mechanism of proton transfer into the aqueous bulk phase in these two systems. The implications for models of localized- delocalized energy coupling by proton gradients will be discussed.

P-540
Electrochemical behaviour of Dps—a mini-ferritin

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Iron regulation is a vital process in organisms and in most of them it is accomplished through the metal solubilisation and storage by ferroxidase enzymes of the Ferritin family, which have the ability of sequester, oxidize and mineralize ferrous ions using oxygen or hydrogen peroxide as substrate. DNA-binding proteins from starved cells (Dps) belong to this Ferritin's family. Dps belongs to the sub-type designated as mini-ferritins and, besides iron storage and release capability, is responsible for hydrogen peroxide resistance showing the ability to form stable complexes with DNA. The preferable co-substrate of this enzyme is H_2O_2 although the reaction can occur in the presence of oxygen with lower rate [1, 2]. In this work, the electrochemical behaviour of the recombinant Dps from *Pseudomonas nautica*, was assessed as a function of metal content in anaerobic environment with H_2O_2 as co-substrate. The obtained electrochemical results together with spectroscopic studies allowed inferring some new hypothesis on the Dps iron uptake mechanism.

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P-541
Myoglobin at Au/SAM junctions: temperature, high pressure and solvent kinetic isotope effects

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We applied electrochemical methodology to study electron exchange and accompanying medium reorganization effects

for myoglobin electrostatically immobilized on Au-deposited mixed self-assembled monolayers (SAMs) of the composition: –S–$(CH_2)_{11}$–COOH/–S–$(CH_2)_{11}$–OH. Our approach allows for a soft switching of the haem group charge state and accurate probing of the accompanying reorganizational dynamics of conformational (quasi-diffusional) and quantum (e.g. proton-related) modes. The electron transfer rate constants were determined with H_2O or D_2O as solvent, under the variable temperature (288-328 K) or pressure (5-150 MPa) conditions, revealing the overall reorganization free energy of 0.5±0.1 eV, activation volume of -3.1±0.1 cm^3 mol^{-1} and inverse solvent kinetic isotope effect of 0.7±0.1 (25 °C). On the grounds of an extended charge-transfer theory, we propose specific proton-coupled ET mechanism additionally coupled to the slow conformational dynamics of the protein matrix accompanied by translocation(s) of haem-adjacent water molecule(s).

P-542
Proton gradients across pore spanning membranes: towards on-chip energy conversion
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In cell organelles, chemiosmotic potentials resulting from proton gradients across membranes are widely used to fix chemical energy in forms of ATP. The high efficiency of this protein-mediated energy conversion raises interest for artificial proton gradient setups. To investigate proton transport across artificial membranes, we prepared pore spanning membranes (PSMs) on porous silicon substrates via *painting* technique. This allowed us to trap aqueous content of well-defined composition and volume inside the substrates microcavities. Nigericin, a peptide that acts as an H^+-K^+-antiporter and bacteriorhodopsin, a transmembrane protein which is well known to be a light driven proton pump, were reconstituted into the pre-formed PSMs to achieve proton transport from one aqueous compartment to the other. Changes of proton concentrations inside the pores were monitored by means of confocal laser scanning microscopy (CLSM). Therefore, pores were filled with pyranine, a pH-sensitive fluorescence dye and variations in intensity were measured to analyze proton translocation. We were able to show that both, nigericin and bacteriorhodopsin are capable of building up a proton gradient across PSMs and plan to co-reconstitute ATP synthases for on-chip energy conversion by formation of ATP.

P-543
Application of the Gibbs free energy profiles to sequential biochemical reactions
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The full understanding of the energetic details of complex metabolic reaction sequences requires a step-by-step analysis of the Gibbs Free Energy (G) changes of the "parasystem" (i.e., a collection of atoms comprising all the molecules participating in a given reaction) as it gradually changes from its initial-reactants state to its final-products state along the reaction pathway. Knowing the respective equilibrium constants of each of the participating reaction steps and also the actual *in vivo* concentrations of the metabolites involved, a free-energy profile can be constructed that will reveal important information about the progress of the reaction as driven by thermodynamic forces. This approach will be illustrated on some biochemical reactions including the glycolytic/gluconeogenetic pathways. Furthermore, the often misleading text-book representation of enzymatic catalysis will be reexamined and explained in thermodynamic terms using the free-energy profiles of both the non-catalyzed and the enzyme-catalyzed reactions.

O-544
Mechanistic insights for redox-active proteins functionalized at Au/SAM junctions
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Redox-active proteins can be diversely functionalized at metal-deposited self-assembled monolayers (SAMs) of a widely variable composition and thickness. The voltammetric methodology in combination with the advanced data processing procedures allow for comprehensive kinetic data within the congruent series of nano-devices and the subsequent calculation of the key physical parameters, such as the medium reorganization energy of ET, the donor-acceptor electronic coupling, effective relaxation time (related to the protein's and environment's fluctuational dynamics), etc. In our studies the "model" redox protein, cytochrome *c* (CytC), was either freely diffusing to SAM terminal groups (mode 1), or attached to SAMs through the electrostatic interaction (mode 2), or specific "wiring" (mode 3). Another redox-active protein, azurin, was confined at terminal SAM groups through the hydrophobic interaction (mode 4). Diverse experimental strategies including the variation of SAM thickness, solution viscosity temperature and hydrostatic pressure, allowed for a severe demonstration of the full adiabatic and nonadiabatic control (thinner and thicker SAMs, respectively), and the intermediary regime, in a nice agreement with the major theoretical predictions.

O-545
Proton transfers in a light-driven proton pump
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Illumination of bacteriorhodopsin causes isomerization of all-trans retinal to 13-cis,15-anti and a cyclic reaction ensues, in which the protein and the chromophore undergo conformational changes with an overall ten millisecond turn-over time and a proton is transported from one membrane side to the other. With crystal structures of six trapped intermediate states and plausible structural models for the remaining two intermediates, structures are now available for the initial

bacteriorhodopsin state and all intermediates. They reveal the molecular events that underlie the light-induced transport: protonation of the retinal Schiff base by Asp85, proton release to the extracellular membrane surface, a switch event that allows reprotonation of the Schiff base from the cytoplasmic side, side-chain and main-chain motions initiated in the cytoplasmic region, formation of a single-file chain of hydrogen-bonded water molecules that conducts the proton of Asp96 to the Schiff base, and reprotonation of Asp96 from the cytoplasmic surface. The observed changes can be summarized as a detailed atomic-level movie in which gradual relaxation of the distorted retinal causes a cascade of displacements of water and protein atoms results in vectorial proton transfers to and from the Schiff base.

P-546

Studying electron transfer in oligopeptides through μSR

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Electron transfer (ET) processes are fundamental in photosynthesis, respiration and enzyme catalysis. The relative importance of superexchange and sequential mechanisms in biological ET is still a matter of debate. The identification of any "stepping stones" necessary for electron hopping is a key point in the understanding of long range ET. Hence, the study of a single event in the sequence of reactions occurring in these phenomena is a fundamental but formidable task.
Muon spin relaxation (μSR) has been shown to be sensitive to charge transport on a molecular lengthscale. The muon is a very sensitive probe of electron transport, as any changes to the electronic density sampled by the muon can change its spin polarization, which can easily be measured. In this context, a very useful tool is the detection of the so-called Avoided Level Crossing (ALC) resonances [1]. The enhancement in the loss of polarization of the muon's spin on these resonances dramatically increases sensitivity.
We show that a laser pump - μSR probe technique can measure ET processes at particular, and most importantly known, sites within the amino acids chain, and therefore track the time evolution of the electron over the molecule.

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P-547

Involvement of histidine in electron and proton transfer in bacterial photosynthetic reaction center

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Keywords: Photosynthesis, reaction center, electron transfer, proton transfer, Fourier transform infrared, L210DN, isotopic labeling, band assignment, histidine, mechanism

In photosynthesis, the central step in transforming light energy into chemical energy is the coupling of light-induced electron transfer to proton uptake. In the photosynthetic reaction center (RC) of *Rhodobacter sphaeroides*, fast formation of the charge separated state $P^+Q_A^-$ is followed by a slower electron transfer from the primary quinone Q_A to the secondary quinone Q_B and the uptake of a proton from the cytoplasm to Q_B. Previous Fourier transform infrared (FTIR) measurements on RC suggested an intermediate X in the $Q_A^-Q_B$ to $Q_AQ_B^-$ transition. Mutation of the amino acid AspL210 to Asn (L210DN mutant) slows down proton uptake and oxidation of Q_A^-. Using time-resolved FTIR spectroscopy we characterized this RC mutant and proposed specific IR bands that belong to the intermediate X. To study the role of the iron–histidine complex located between Q_A and Q_B, we performed fast-scan FTIR experiments on the L210DN mutant marked with isotopically labelled histidine. We assigned IR bands of the intermediate X between 1120 cm^{-1} and 1080 cm^{-1} to histidine vibrations. These bands show the protonation of a histidine, most likely HisL190, during the $Q_A^-Q_B$ to $Q_AQ_B^-$ transition. Based on these results we propose a new mechanism of the coupling of electron and proton transfer in photosynthesis.

O-548

Sodium influence on energy transduction by bacterial complexes I

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Complex I of respiratory chains is an energy transducing enzyme present in most bacteria and in all mitochondria. It is the least understood complex of the aerobic respiratory chain, even though the crystallographic α-helical structures of bacterial and mitochondrial complexes have been recently determined [1-2]. This complex catalyses the oxidation of NADH and the reduction of quinone, coupled to cation translocation across the membrane.
Rhodothermus marinus complex I, our main model system, is a NADH:menaquinone oxidoreductase and has been extensively characterized. We have made an exhaustive study in order to identify all the subunits present in the complex [3]. The nature of the coupling charge of *R. marinus* complex I was investigated using inside-out membrane vesicles, which were active with respect to NADH oxidation and capable of creating and maintain an NADH-driven membrane potential ($\Delta\Psi$) positive inside. It was observed that this bacterial complex I is able of H^+ and Na^+ transport, although to opposite directions. The coupling ion of the system was shown to be the H^+ being transported to the periplasm, contributing in this way to the establishment of the electrochemical potential difference, while Na^+ is translocated to the cytoplasm [4]. The sodium ion extrusion from the membrane vesicles was due to the activity of complex I, since it was sensitive to its inhibitor rotenone, and it was still observed when the complex I segment of the respiratory chain was isolated by the simultaneous presence of cyanide and external quinones. Additional studies have shown that although neither the catalytic reaction nor the establishment

of the ΔpH requires the presence of Na^+, the presence of this ion increased the proton transport. Combining all these results, a model for the coupling mechanism of complex I was proposed, suggesting the presence of two different energy coupling sites, one that works only as a proton pump (Na^+ independent), and the other functioning as a Na^+/H^+ antiporter (Na^+ dependent) [4]. This model was reinforced by further studies performed in the presence of the Na^+/H^+ antiporter inhibitor, 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA) [5].

A deeper inside into the coupling mechanism of this enzyme was provided by studying the influence of sodium ions on energy transduction by complexes I from *Escherichia coli* and *Paracoccus denitrificans*. It was observed that the Na^+/H^+ antiporter activity is not exclusive of *R. marinus* complex I, since the *E. coli* enzyme is also capable of such a transport, but is not a general property given that the *P. denitrificans* enzyme does not performed sodium translocation [6]. Due to the fact that *R. marinus* and *E. coli* enzymes reduce menaquinone while *P. denitrificans* complex I reduce ubiquinone, it is suggested that the Na^+/H^+ antiporter activity may be correlated with the type of quinone used as substrate.

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O-549

Mechanism of proton transfer in nitric oxide reductase: computational study

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Under anaerobic conditions some bacteria can use nitrate instead of oxygen in a process called denitrification. During denitrification, the reduction of NO to N_2O is catalyzed by a membrane-bound enzyme nitric oxide reductase (NOR). This enzyme is an important step in the evolution of a respiratory system: NOR belongs to the superfamily of O_2-reducing heme-copper oxidases and is assumed to be the evolutionary ancestor of cytochrome c oxidase. The understanding of NOR functioning was limited by the lack of structural information, but recently the first structures (cNOR type from *Ps. aerug.* and qNOR type from *G. stearoth.*) were solved [1-2].

We will present results of the first computational studies of NOR (both cNOR and qNOR types) [2-3]. The studies include: **(*i*)** large-scale all-atom MD simulations of proteins in their natural environment (i.e. embedded in membrane and solvent), which were performed to describe water dynamics inside the protein and to identify potential proton transfer pathways, and **(*ii*)** free-energy calculations by the empirical valence bond (EVB) method [4] for the explicit proton translocations along the pathways established by MD. Among important findings are new proton pathways, which were not predicted from the X-ray structure and could be identified only by means of computer simulations. Simulations also reveal that, despite a high structural similarity between cNOR and qNOR, these enzymes utilize strikingly different proton uptake mechanisms. Our results provide insights into the functional conversion between NO and O_2 reductases, and into evolution proton transfer mechanisms and respiratory enzymes in general.

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P-550

Functional and structural characterization of cytochrome c_6-like from a nonphotosynthetic organism

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The genome of the bacterium *Geobacter sulfurreducens* (*Gs*) encodes for 111 *c*-type cytochromes (1). Genetic studies using cytochrome deficient *Gs* strains and proteomic studies identified cytochromes that were produced under specific growth conditions (2-4). A putative outer-membrane cytochrome, OmcF, is crucial for Fe^{3+} and U^{6+} reduction and also for microbial electricity production (4). OmcF is a monoheme *c*-type cytochrome with sequence similarity to soluble cytochromes c_6 of photosynthetic algae and cyanobacteria (4). The structure of oxidized OmcF was determined (5) being the first example of a cytochrome c_6-like structure from a non-photosynthetic organism. The structural features of OmcF hinted a different function compared to cytochromes c_6 from photosynthetic organisms, being an excellent example of how structurally related proteins are specifically design by Nature to perform different physiological functions. In order to elucidate OmcF structural-functional mechanism, isotopic labeled protein (^{15}N and ^{13}C) was produced and its structure in the reduced form determined by NMR. Single point-mutations at key residues were produced by site-directed mutagenesis and their impact on the structural and functional properties of OmcF will be presented.

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Acknowledgments

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P-551

The no-synthase paradox

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Keywords: Nitric Oxide, NO-Synthase, Molecular Mechanism, Biophysics, Kinetics

In the early 90s, the search for the source of nitrogen monoxide (NO) production in mammals led to the discovery of three major isoforms of NO-Synthases (NOS): the neuronal NOS (nNOS), the inducible NOS (iNOS), and the endothelial NOS (eNOS) ((1)). 20 years later, based on genomic analysis, numerous NOS-like proteins have been identified in the genome other organism and in particular of several bacteria (*Bacillus Anthrax*, *Staphylococcus aureus*… (2)).

In spite of superimposable 3D structures and the ability to catalyse NO production, all these enzymes are carrying different (if not opposite) physiological activities including cGMP signalling, cytotoxic activities, anti-oxidant defence, metabolism… Moreover, NOSs become increasingly associated to oxidative-stress related pathologies ranging from neurodegenerative disorders, cardiovascular and inflammatory diseases, diabetes, cancers (3)….

This apparent paradox seems related to the belief that the strong similarity of sequence and structure of NO-Synthases must lead to a unique and identical functioning (NO production) for all isoforms. This is blatant for bacterial NOS-like proteins that are lacking the essential components required for NO biosynthesis but remain considered as genuine NO synthases. This approach might remain an obstacle to the understanding of NOS actual biological role and could prevent the design of efficient NOS-targeted therapeutic strategies.

To elucidate this "NOS paradox" our group has initiated a multidisciplinary approach that aims to relate the wide diversity of NOS biological activities to variations in the catalytic mechanism of NOSs, to modifications of their regulation patterns and to adaptations to their physiological environment. In this context we have been investigating the mechanism of bacillus subtilis NOS-like proteins with a special focus on the features that are specific to NOS mechanism*: i)* electron and proton transfers and the role of the substrate and the pterin cofactor *ii)* oxygen activation and the role of the proximal ligand *iii)* the very molecular mechanism and the variations in the nature of reaction intermediates.

For that matter we have been using a combination of radiolytical techniques (cryoreduction with ^{60}Co y-irradiation, pulse-radiolysis with the ELYSE electron accelerator), state-of-the-art spectroscopies (EPR, ATR-FTIR and resonance Raman, and picoseconds UV-Visible absorption spectroscopies), organic synthesis (synthesized substrates and cofactors analogues) biochemistry and molecular biology (site-directed mutagenesis).

We will present our results on the coupling of electron and proton transfers, on the tuning of the proximal "push effect" and we will discuss the conditions that favour for each NOS isoform NO production versus other reactive nitrogen and oxygen species.

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O-552

Molecular characterization of photosynthetic iron oxidation

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Photosynthetic Iron Oxidation (PIO) is an ancient form of photosynthesis with relevant consequences in the shaping of the planet. This form of metabolism may have been involved in the deposition of geological structures known as Banded Iron Formations, which hold key information regarding the co-evolution of photosynthesis and Earth. *Rhodopseudomonas palustris* TIE-1 and *Rhodobacter ferroxidans* SW2 both use ferrous iron as an electron donor to support photosynthetic growth (*i.e.* photoferrotrophy). The SW2 *foxEYZ* operon can stimulate light-dependent iron oxidation by other bacteria. It codes a two-heme cytochrome, a pyrroloquinoline quinone protein and an inner membrane transporter, respectively. In TIE-1 the *pioABC* operon is required for photoferrotrophy. It codes for a ten-heme cytochrome, an outer membrane beta-barrel and a high potential iron-sulfur protein (HiPIP) respectively. Here we present functional and structural characterization of proteins involved in PIO. This molecular characterization is essential for understanding this mode of bioenergetic metabolism, and may one day aid the development of biotechnological applications like microbial fuel cells and bioremediation.

P-554
Anoxia induces increased activity of alternative oxidase in fungus *Phycomyces blakesleeanus*
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Alongside classical, cytochrome respiratory pathway, *Phycomyces blakesleeanus* possess alternative, cyanide-resistant respiration (CRR) facilitated by alternative oxidase (AOX). In order to study role of oxygen in regulation of CRR, the effects of cyanide on respiration of 24h old mycelia in aerated (control), hypoxic and anoxic conditions were measured. Mycelium was incubated in these conditions for 1.5h, 3h and 5h. After 1.5h, AOX activity was increased only in specimens incubated in anoxic conditions (13.6%). After 3h, increase in AOX activity was significant in both hypoxic and anoxic specimens (18.9% and 18.8%, respectively), with even greater increase after 5h, 20.7% for hypoxic and 23.3% for specimens in anoxic conditions. Mycelia treated for 5h was then oxygenated for 10 minutes. This induced decrease in AOX activity of 17% in anoxic and even 23.9% in hypoxic mycelia. AOX is recognized as one of the mechanisms for maintaining low levels of reduced ubiquione which can function in conditions in which cytochrome chain is disabled, such as anoxia. This is in concordance with results obtained on *P. blakesleeanus*, where AOX levels rise in hypoxic and anoxic conditions and decrease close to control level shortly after introduction of oxygen into the system.

P-555
Influence of *Escherichia coli* F_0F_1-ATPase on hydrogenase activity during glycerol fermentation
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E. coli encodes four hydrogenases (Hyd); only three of these, Hyd-1, Hyd-2 and Hyd-3 have been well characterized. Hyd-2 has been shown recently to reversibly evolve hydrogen during glycerol fermentation at pH 7.5 [1]. Proton reduction was inhibited by N,N'dicyclohexylcarbodiimide suggesting a link with the proton-translocating F_0F_1-ATPase. Indeed, at pH 7.5 in an *E. coli* mutant (DK8) lacking F_0F_1 overall Hyd-activity was reduced to approximately 50% of the wild type activity; Hyd-2, but not Hyd-1, was detected in an in-gel activity assay. F_0F_1 is therefore suggested to be required for Hyd-1 activity. At pH 6.5 in glycerol medium Hyd-activity in DK8 was $\sim$10% of wild type activity and Hyd-1 and Hyd-2 exhibited only weak activity. This indicated a significant F_0F_1 contribution towards Hyd-activity as pH decreased. Furthermore, at pH 5.5 Hyd-activity was negligible and only a very weak activity band corresponding to Hyd-2 could be observed. These results suggest that F_0F_1-ATPase is essential for hydrogenase activity during glycerol fermentation at pH 5.5. Taken together, the results suggest an interdependence between Hyd-1, Hyd-2 and F_0F_1-ATPase activity.

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O-557
Characterization of a vacuolar cytochrome b561 by redox titration and spectrum analysis
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Cytochromes *b*561 (Cyts-b561) of invertebrates, vertebrates and plants are predicted to consist of six *trans*-membrane helices, with four conserved histidines involved in the coordination of two hemes. They are generally accepted to catalyze ascorbate-driven *trans*-membrane electron transport. One isoform from the tonoplast of plant cells (TCytb) has been expressed in yeast and the characterization of the recombinant TCytb has just been started[1,2].
We have titrated the Q-band spectra of the wild type, H83A/H156A, and H83L/H156L TCytb at room temperature with different reducing agents. Earlier results have proven that these mutants contained only one heme. The series of spectra were analized by SVD. Fitting the V vectors by the Nernst equation allowed us (1) to determine the difference spectra characterizing each of the two hemes, and (2) to show that the double mutant TCytb-s have only the low potential heme, in good agreement with earlier results obtained at low temperature and/or by different spectroscopy techniques[1,2].

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Ion channels and disease

O-558
hERG1 channels: from antitargets to novel therapeutic targets in oncology.
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Ion channels and transporters control many facets of cancer cell biology[1] and blocking the activity of these impairs tumor cell growth *in vitro* and *in vivo*. This new paradigm has opened new opportunities for pharmaceutical research in oncology[1,2]. We have contributed to this field showing that

K_v11.1 (hERG1) channels are aberrantly expressed in several human cancers where they control different aspects of the neoplastic cell biology such as proliferation and apoptosis, invasiveness and angiogenesis, the latter through the regulation of VEGF secretion (reviewed in[1]). The hERG1-dependent effects were shown *in vitro* and, more recently, *in vivo*. In preclinical models of both leukemia[1] and colorectal cancer[3], hERG1 overexpression confers a higher malignancy to neoplastic cells. Moreover, hERG1 blockers have therapeutic potential, since preclinical tests showed that treatment with specific hERG1 blockers overcame chemoresistance in acute leukemias[4] as well as reduced GI cancer growth, angiogenesis and metastatic spread[3]. The overall message emerging from our data is that the hERG1 protein represents a novel biomarker and drug target in oncology. Up to now, hERG1 was considered an "antitarget" due to the cardiac side effects that many (but not all) hERG1 blockers produce and that result in lengthening the electrocardiographic QT interval. We report here recent studies on known and newly developed hERG1 blockers that exhibit no cardiotoxicity and are more specific for the hERG1 channels expressed in cancer cells.

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O-559

Analysis of the K^+ current in human T cells in hypercholesterinaemic state

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We reported previously that the increase of the cholesterol content of the cell membrane (*in vitro*) modified the biophysical parameters of the gating of Kvl.3 K^+ ion channels in human T-lymphocytes. In our present study we aimed to determine the effect of hypercholesterolemia on the biophysical parameters of Kv1.3gating and the proliferation of T cells.

T-lymphocytes were isolated from the peripheral blood of patients with cholesterol level considered normal (<5.2 mmol/l,control group) and patients with hypercholesterinaemia (hc). Whole-cell K+ currents were measured in patch-clamped T cells and the kinetic (activation and inactivation kinetics) and equilibrium parameters (voltage-dependence of steady-state activation) of Kv1.3 gating were determined. Lymphocyte proliferation was measured using CFSE staining with and without anti-CD3 and anti-CD28 stimulation.

Our results indicate that the biophysical parameters of Kv1.3 gating are similar in the control group and in the 'hc' samples. The CFSE-based assay showed that hypercholesterolemic T cells had higher spontenaous activation rate compared to control group. However, T cells from high cholesterol level patients challenged by anti-CD3 and anti-CD28 exhibited lower proliferation rate than control cells.

P-560

Endothelial cells involved in chronic thromboembolic pulmonary hypertension have altered calcium homeostasis

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Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare and late possible consequence of venous thromboembolism. In this study, cellular properties of endothelial cells isolated from surgical material of CTEPH patients (CTEPH-hECs) were compared with control human pulmonary artery endothelial cells (hPAEC).

The purified endothelial cells from CTEPH surgical tissue and the control hPAECs were cultured under standard conditions. CTEPH-hECs and hPAECs were stained for von Willebrand factor and vascular-endothelial cadherin. To address the intracellular calcium changes, Fura-2/am loaded cells were challenged with 10μM histamine, intracellular calcium stores were depleted with 15μM BHQ and the activation of transient receptor potential canonical channels (TRPCs) were followed.

CTEPH-hECs and hPAECs both express similar pattern of von Willebrand factor and vascular-endothelial cadherin. CTEPH-hEC show no significant differences in basal calcium level (140,89±51,40 mM Ca^{2+} for CTEPH-hEC, n=221; 128,61±49,16 for hPAEC, n=245) as well as in the histamine-induced calcium peak (278,78±21,97 for CTEPH-hEC, n=110; 242,40±7,65 for hPAEC, n=133) in comparison to control hPAECs. Interestingly, the challenge with BHQ leads to a significant increase in calcium store depletion signal (505,27±37,51 for CTEPH-hEC, n=111; 200,67±25,77 for hPAEC, n=112). By adding back the calcium to the cells a significant difference in the calcium influx occurs (652,39±46,88 for CTEPH-hEC, n=111; 322,84±19,33 for hPAEC, n=112).

The isolated and purified endothelial cells from surgical material of CTEPH patients present the characteristic endothelial phenotype, but they show altered calcium homeostasis at the stored calcium and store operated calcium influx level.

P-561
Identification of an intramolecular disulphide bond in the Na-channel β1-subunit
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Generalized epilepsy with febrile seizures plus (GEFS+, OMIM 604233) is a childhood genetic epilepsy syndrome correlated to mutations in the ancillary β-subunit of neuronal voltage-gated sodium channels (NaChs). β1-subunit is non-covalently associated with NaCh α- subunits, serving as modulator of channel activity, regulator of channel cell surface expression and as cell adhesion molecule. The first and best characterized GEFS+ mutation is the C121W. This mutation changed a conserved cysteine residue into a tryptophan, disrupting a putative disulphide bridge that should normally maintain an extracellular immunoglobulin-like fold.
In this study, we investigated the presence of this putative disulphide bond using 2-D-diagonal-SDS-PAGE, where the proteins were separated in the first dimension in absence of a reduction agent and in presence of it in the second dimension. This method allows to visualize the protein above the diagonal experimentally confirming that the disulphide bond is intramolecular. The protein spot was in-gel digested and identified by MS/MS. This data support the hypothesis of a loss of the S-S bond causing a molecular defect on GEFS+ β1-mutation.
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O-562
Calcium channels of vascular remodelling
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Vascular remodelling in physiology and disease is substantially due to vascular smooth muscle cells and endothelial cells that switch to phenotypes characterised by proliferation, migration, secretion, invasion etc. We have sought to understand ion channels in this context, particularly in human diseased tissue. Data are consistent with roles of the potassium channels KCa3.1 and Kv1.3. Most of our recent work has, however, focused on calcium-permeable channels that are chemically activated. The data suggest that the TRPC1 channel subunit has an important role and is up-regulated in remodelling. We showed, for example, that TRPC1, operating with TRPC5, is stimulated by oxidized phospholipids (AL-Shawaf et al 2010, *ATVB* 30, 1453-). Intriguingly TRPC1 inhibits the function of other TRPC channels and so it is unclear how it acts to drive calcium entry. Other types of calcium channel also contribute: Data suggest roles for Orai1 and STIM1 as subunits/regulators of CRAC channels. Growth factor (PDGF and VEGF) signalling shows striking dependence on such channels, conferring functional significance for cell migration and angiogenesis (Li et al 2011, *Circ Res* published on-line). These and other recent findings will be discussed. Financial support is provided by Wellcome Trust, BHF, MRC.

P-564
LQTS acquired by pentavalent antimony treatment in guinea pigs
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Pentavalent antimony (PA) is the mainstream agent of choice for Leishmaniasis treatment. In therapeutic doses the PA treatment has cardiac side effects that include QT interval prolongation, torsade de pointes arrhythmias and sudden death. The objective of this study was to investigate arrythmogenic properties of PA in treated guinea-pig, analyzing ECG parameters, ventricular action potential and I_{Kr}, I_{ks} ionic currents. The guinea-pigs received daily 20mg/kg PA or not for 15 days. PA group had an increased QRS complex (control=10 ± 3 and PA=20±6, $p<0,01$), QTc interval duration (control=270±5 and PA=305±5, $p<0,01$) and QT dispersion (control=29±6 and PA=54±5, $p<0,05$) on ECG. The AP analysis demonstrated triangulation (control=41±9 and PA=83±15, $P<0,01$) and prolongation at 30% (control=55±1 and PA=85±3, $p<0,05$) and 90% (control=100±3 and PA=168±5) AP with PA treatment. Also, the AP treatment reduced markedly IKr density (control=2,1±0,7 and PA=0,6±0,2, $p<0,01$) and do not alter IKs density (control=5,5±1,5 and PA=5,9±1,4, $p<0,01$). The PA treatment was lethal in 30% of the animals. Our results suggested that PA is a proarrhythmic drug that upon chronic use may improve the mortality and cause repolarization disturbances that characterized acquired long QT syndrome.

O-565
Voltage-gated ion channel dysfunction precedes cardiomyopathy development ion the dystrophic heart
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Duchenne muscular dystrophy (DMD) is associated with severe cardiac complications. Recent research suggests that impaired voltage-gated ion channels in dystrophic cardiomyocytes accompany cardiac pathology. It is, however, unknown if ion channel defects are primary effects of dystrophic gene mutations, or secondary effects of the developing cardiomyopathy. Here, we studied Na and Ca channel impairments in dystrophic neonatal cardiomyocytes, derived from DMD mouse models, prior to cardiomyopathy development. Dystrophin-deficiency reduced Na current density. In addition, extra utrophin-deficiency altered Na channel gating. Moreover, also Ca channel inactivation was reduced, suggesting that ion channel abnormalities are universal primary effects of dystrophic gene mutations. To assess developmental changes, we also studied Na channel impairments in dystrophic adult cardiomyocytes, and found a stronger Na current reduction than in neonatal ones. The described Na channel impairments slowed the action potential upstroke in adult cardiomyocytes, and only in dystrophic adult mice, the QRS interval of the ECG was prolonged. Ion channel impairments precede pathology development in the dystrophic heart, and may be considered cardiomyopathy triggers. Supported by Austrian FWF (P19352).

P-566

Exercise does not prevent cardiac autonomic dysfunction in rats treated with anabolic steroids

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Keywords: Anabolic androgenic steroids, regular exercise, heart rate variability, cardiac autonomic dysfunction

The aim of this study was to evaluate the changes in HRV induced by administration of nandrolone decanoate (ND), with or without exercise during 8 weeks(W). 40 male rats were divided into four groups: sedentary (DS) and trained (DT) treated with ND; sedentary (CS) and trained (DT) treated with vehicle. Exercised were carried 5 days/W. ECG was recorded at 0, 4^{th} and 8^{th}W. Time- and frequency-domain analysis of heart rate variability was performed. SDNN and RMSSD were reduced in DT (7.81±2.85ms) and DS (6.21±1.13ms) compared to CS (9.6±2.6ms) after 4^{th} and 8^{th}W (DT:7.02±3.17 ms, DS:4.6±1.12 ms vs CS:8.4±1.1 ms); pNN5 was reduced only after 8^{th}W (DT:23.3±5.7ms, DS:14.3±6.3ms vs CS:33.2±8.5ms). CT showed increase in RMSSD and pNN5% after 8W, compared to CS (RMSSD=10.47±2.36ms vs 8.38±1.1ms; pNN5%=43.2±9 vs 33.2±8.5). High frequency(HF) of DS and DT was reduced compared to CS after 4^{th} (6.65±6ms^2, 4.9±1.7ms^2 vs 12.8±2.63ms^2) and 8^{th} (5.34±3.39ms^2, 4.16±0.93ms^2 vs 11.54 ± 1.6ms^2) while LF/HF ratio increased after 4^{th} (2.1±1.3, 1.95±0.83 vs 0.9±0.2) and 8^{th} (2.08±1.0, 2.5±0.44, 0.85±0.14). CT had a rise in HF compared to CS on 8^{th}W (14.75±2.44ms^2 vs 11.5±1.6ms^2). A Supraphysiological dose of ND induces cardiac autonomic dysfunction and regular exercise does not prevent this effects.

P-567

Mg^{2+}-inhibitable TRPM7-like cation current in human atrial myocytes

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The aim of our work was to identify the presence of TRPM7-like current in human atrial myocytes, and to investigate the potential role of these channels in the electrical activity of atrial cells during atrial fibrillation (AF). Myocytes were isolated from right atria biopsies from 72 adult patients in sinus rhythm (SR) and 49 patients in AF. We demonstrate that in both patient groups under voltage clamp, with voltage-dependent and other ion channels inhibited, cells dialyzed with low intracellular free $[Mg^{2+}]_i$ produced outward-rectifying TRPM7 currents that could be suppressed by raising extracellular $[Mg^{2+}]_o$ or by application of Gd^{3+} and 2-APB (at 100 μM), known to block TRP channels. The current was absent in cells dialyzed with physiological free $[Mg^{2+}]_i$ or MgATP, but that did not protected from the spontaneous run-up in divalent free external solutions. In addition, we established that the density of activated TRPM7-like current in cells from patients with AF was higher as compare with those with SR (5.30±0.39 and 3.62±0.23 pA/pF, respectively, at +80mV, $p<0.001$). In conclusion: this is the first study, which describes TRPM7-like current in native human atrial cardiomyocytes that operates under pathophysiological conditions.

Acknowledgements
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O-568

Clustering of voltage-gated sodium channel $Na_V1.8$ in lipid rafts is essential for action potential propagation in nociceptive unmyelinated axons

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It has been over 15 years since sensory neuron-specific sodium channel $Na_V1.8$ was identified. Since then $Na_V1.8$ has been shown to play a crucial role in pain pathways, and it became a prominent drug target for novel pain killers. In contrast to myelinated neurons, mechanisms that target voltage-gated sodium channels to the unmyelinated C-fibre axons are largely unknown. We investigated the localisation of $Na_V1.8$ in unmyelinated primary sensory neurons. $Na_V1.8$ was found to be clustered in lipid rafts on unmyelinated axons. When the lipid rafts are disrupted, remarkable reduction in both the conduction velocity and the number of cells responsive to mechanical stimuli to the unmyelinated axons were seen. Using a compartment culture system, we also found that disruption of rafts in the middle region of the sensory axons caused a significant reduction in the responsiveness of the neurons to chemical stimuli to nerve endings. This is due to the failure of action potential propagation through the axons. These data suggest that clustering of $Na_V1.8$ in lipid rafts of unmyelinated fibres is a key factor for the functional properties of the channel, which may due to a change in the voltage threshold. Disruption of the $Na_V1.8$ cluster and modifying lipid rafts in primary sensory neurons may be a useful new approach to control the excitability of nociceptive neurons.

P-569

Effect of ion channel blockers on T-lymphocytes stimulated with anti-CD3 and anti-CD28

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Ion currents in are crucially important for activation of T-lymphocytes. Our aim was to investigate how the blockage of various ion channels in isolation or in combination affects mitogen-dependent activation and proliferation of T-cells.
We activated human peripheral blood lymphocytes using monoclonal antibodies against the TCR-CD3 complex and CD28. We applied specific channel blockers inhibiting the major ion channels of the T-cell: either the Kv1.3 (TEA or Anuroctoxin), IKCa1 (TRAM-34), or the CRAC channel (2-APB) alone or in combination. Five days after the stimulus we measured the change in cell size and cellular granulation with

flow cytometry along with the proportion of dividing cells, using CFSE (carboxyfluorescein succinimidyl ester) dilution assay. Our measurements indicated that the ion channel blockers suppressed the proportion of dividing cells in a dose-dependent manner. Increasing the strength of the stimulation reduced the potency of the blockers to inhibit cell proliferation and eventually the blockers were ineffective in decreasing lymphocyte proliferation. We experienced the greatest amount of inhibition using the combination of the blockers, which indicates synergy in the regulation pathway of the various ion channels.

P-571

Quantitative NaPi2b transporter expression in different histomorphological types of ovarian cancer

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Recently, sodium dependent phosphate transporter NaPi2b was revealed as potential marker for breast, thyroid and ovarian cancer. In vivo, NaPi2b is involved in maintenance of phosphate homeostasis and mutations or aberrant expressions of its gene (SLC34A2) are associated with several diseases including cancer. However, data about NaPi2b mRNA expression in different types of cancer and correspondent normal tissues are controversial and restricted.
We investigated *SLC34A2* gene expression level in normal ovarian tissues and different histomorphological types of ovarian tumors using real-time PCR analysis. It was found that *SLC34A2* gene was highly expressed in well-differentiated endometrioid and papillary serous tumors, but was not expressed in low-differentiated tumors, benign tumors and in most normal tissues. mRNA expression of *SLC34A2* in serouse and endometrioid ovarian tumors accurately correlated with protein expression that was detected in these tumor samples by Western blot analysis and immunohistochemistry in our previous investigation.
Upregulation of *SLC34A2* gene expression in well-differentiated tumors may reflect cell differentiation processes during ovarian cancerogenesis and could serve as potential marker for ovarian cancer diagnosis and prognosis.

O-572

Membrane-permeant inhibitors of the potassium channel Kv1.3 induce apoptosis in cancer cell lines

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Potassium channels are emerging therapeutic targets in cancer. Kv1.3 has been shown to play an important regulatory role during proliferation and apoptosis. In lymphocytes and other cells Kv1.3 is present in both the plasmamembrane and the inner mitochondrial membrane (mitoKv1.3). Inhibition of mitoKv1.3 by pro-apoptotic Bax results in production of reactive oxygen species and leads to cytochrome c release and death. Three distinct membrane-permeant inhibitors of Kv1.3 induce apoptosis in different cancer cell lines expressing mitoKv1.3, while membrane-impermeant inhibitors do not induce cell death, suggesting that apoptosis involves inhibition of mitochondrial and not of plasma membrane Kv1.3. The effect of these drugs is abolished when Kv1.3 is absent or its expression is reduced by siRNA, demonstrating Kv1.3-specificity of the inhibitors. Most importantly, the drugs are able to induce death in two cell lines lacking Bax and Bak. Our work thus identifies a novel pharmacological tool to induce apoptosis in mitoKv1.3-expressing cells and in vivo experiments are under way to test sensibility of tumors to these drugs.

New and notable

P-574

Protein Dynamical Transition and Resolution Effects on Mean Square Displacement in Lysozyme Systems

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In the present contribution a procedure for molecular motion characterization based on the evaluation of the Mean Square Displacement (MSD), through the Self-Distribution Function (SDF), is presented. It is shown how MSD, which represents an important observable for the characterization of dynamical properties, can be decomposed into different partial contributions associated to system dynamical processes within a specific spatial scale. It is shown how the SDF procedure allows us to evaluate both total MSD and partial MSDs through total and partial SDFs. As a result, total MSD is the weighed sum of partial MSDs in which the weights are obtained by the fitting procedure of measured Elastic Incoherent Neutron Scattering (EINS) intensity. We apply SDF procedure to data collected, by IN13, IN10 and IN4 spectrometers (Institute Laue Langevin), on aqueous mixtures of two homologous disaccharides (sucrose and trehalose) and on dry and hydrated (H_2O and D_2O) lysozyme with and without disaccharides. The nature of the *dynamical transition* is highlighted and it is shown that it occurs when the system relaxation time becomes shorter than the instrumental energy time. Finally, the bioprotectants effect on protein dynamics and the amplitude of vibrations in lysozyme are presented.

P-575

DNA damage in breast cancer cells treated with combination of quercetin and anticancer drugs

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Quercetin (Q) is a natural flavonol present at high levels in numerous of plants. It has been epidemiologically associated with bioactive protective effects, but has also been shown to be mutagenic and toxic in *in vitro* cell systems.

We evaluated Q for genotoxicity in MCF-7 breast cancer cells in the presence or absence of doxorubicin (DOX), docetaxel (DTX) and paclitaxel (PTX), anticancer drugs commonly used in chemotherapy of different solid tumors. Damage to DNA was determinated by comet assay. The cells, after treatment with investigated compounds, were washed up and cultured in fresh medium for 0, 24, 48, 72 and 96 hours. We have found that Q by itself caused significant DNA damage. Moreover flavonol enhanced genotoxic effect of anticancer drugs. The highest amount of DNA in the comet tail was observed 48 h after treatment with combination of Q with DOX. Similar changes were found in cells incubated with combination of Q with taxanes – PTX or DTX. However, damage to DNA in this case was considerably lower than damage caused by combination of Q with DTX.
Our results confirmed anticancer and genotoxic activity of quercetin, which makes it a promising candidate for a potential use as a modulator of cytotoxicity and anticancer activity of anthracycline and taxane chemotherapeutics.

P-576
Non-ionizing electromagnetic-own-signal-treatment
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Although the health effects of low-frequency and intensity electromagnetic fields (LFI-EMFs) are controversial, increasing evidence suggests that LFI-EMFs are capable for initiating various healing processes. Many (bio)physical ideas were suggested to explain the influence of LFI-EMFs in living systems but the main effect of LFI-EMFs on cell functions remains vague. However, some effects of LFI-EMFs may be explained by redox and membrane processes. D*uring* diseases, *cells* not only demonstrate altered biochemical processes but also produce altered non-linear bioelectromagnetic complex patterns. Thus, it is reasonable to use non-linear bioelectric and bioelectromagnetic signals from cells of the body for potential therapeutic applications that may be more effective than the artificial LFI-EMFs signals. Our novel EMOST (Electromagnetic-Own-Signal-Treatment) method is based on the utilization of the non-linear, bioelectric and bioelectromagnetic signals of the patients without any electromagnetic wave modulation and inversion of recorded output signals of subjects. Here, we report our some restorative results after EMOST application. We also suggest that the possible effects of the EMOST may be achieved via redox-related processes.

P-577
Macroscopic properties of phospholipid vesicles with domains in phase-separated membranes
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Ternary mixtures of a high-melting lipid, a low-melting lipid, and cholesterol are known to form domains of a liquid-ordered and a liquid-disordered phase in bilayer membranes. Giant vesicles, prepared from a sphingomyelin/dioleoylphosphocholine/cholesterol mixture, are examined using fluorescence microscopy. NBD-labeled lipid and BODIPY-labeled cholesterol are used to identify the phase domains of the membrane. A vesicle with only two domains is chosen because of its simple geometry, for convenient comparison of the experimental results with the theoretical predictions. The volume of the vesicles is gradually decreased and/or increased by changing the osmolarity. The relevant energy terms of the membrane mechanics are the bending energy and the Gaussian bending energy, and the energy of the domain boundary. The energy of the domain boundary is proportional to its length. At the boundary between the domains a contact angle is taken into consideration. In order to obtain the values of the lateral tension and the contact angle, the areas of the domains and the characteristic dimensions of the shape are determined for different volumes. The best fits were obtained for a line tension of 6 ± 3 pN and a contact angle of 1.4 ± 0.3 rad.

P-579
Fractal avalanche ruptures in biological membranes
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Previously, membrane rupture dynamics has been studied in bilayer vesicles under tensile stress, which consistently produces circular pores. We observed very different rupture mechanics in bilayer membranes spreading on solid supports: the ruptures proceeded in a series of rapid avalanches causing fractal membrane fragmentation. The intermittent character of rupture evolution and the broad distribution in avalanche sizes is consistent with crackling-noise dynamics. Such noisy dynamics appear in fracture of solid disordered materials, in dislocation avalanches, in plastic deformations, and domain wall magnetization avalanches**.** We also observed similar fractal rupture mechanics in spreading cell membranes.

O-580
Background K^+ channel gating and cell excitability
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Background or leak potassium conductances are a major determinant of resting potential and input resistance, two key components of cell excitability. These currents are not passive but finely tuned and adapted to cell specific functions. K_{2P} channels producing these currents are tightly regulated by a variety of chemical and physical stimuli including temperature, membrane stretch, free fatty acids, pH, Ca^{2+}, neurotransmitters and hormones as well as protein partners. These different stimuli converge on gating mechanisms that show remarkable conservation between intracellular K2P

channels (TWIK1 channels) and K2P channels located at the plasma membrane (TREK1/2 channels).

P-581
Living at the edge: volume 2 the conduction system of interfacial forces into the alveolar type II cell
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Many epithelia have contact with air-liquid interfaces (A_{LI}). This applies particularly to the lung, where the surfactant secreting alveolar type II cells (AT II) even project into the air. In previous investigations, using new microscopic approaches, we found that the presence of an A_{LI} leads to a paradoxical situation: it is a potential threat that may cause cell injury, but also a important stimulus: AT II cells respond promptly, and show sustained Ca^{2+}-signals that activate exocytosis. Exocytosed surfactant, in turn, clearly prolonged the time to irreversible cell damage, and may be an adaptive and evolutionary defense mechanism against the harmful nature of surface forces. Recently we published that AT II cells are sensing the A_{LI} but how this stimulus is conducted and converted into the cell, is still obscure.
Currently we are searching for potential calcium sources and it seems that the cells signal Ca^{2+} by extracellular Ca^{2+} entry probably through mechanosensitive channels. Special-designed gene chips allowed a whole genome profiling of A_{LI} exposed single cells. These cells react with rapid changes on the transcriptional level: cellular pathways that are involved include e.g. defense response and lipid metabolism, and we identified genes associated with several lung diseases and injuries. We summarize, interface forces are strong, they are acting on the cells and triggering cellular events that are closely related with classical concepts in mechanotransduction, it is very plausible that those forces play a crucial role in the lung surfactant homeostasis.

Abbreviations:
Alveolar type II cell AT II cell
Air-liquid-interface A_{LI}

O-582
Get a grip: engineering ultra-stable interactions to proteins for biophysics and medicine
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Achieving strong and specific binding to proteins is important for many areas of biophysics and also for new therapeutics. The mammalian immune system usually addresses this challenge within a few weeks, but the antibodies raised have modest affinity. We have designed stronger interactions, through chemical and bioengineering approaches. For binding endogenous proteins, we have generated electrophilic affibodies, for proximity-induced covalent binding. For binding peptide tags, we have designed genetically-encoded peptides based on the human pathogen Streptococcus pyogenes that spontaneously form amide bonds to their protein partner. For binding to small-molecule ligands, we have modified the streptavidin-biotin interaction to achieve even stronger biotin binding and mechanical strength. These technologies have been applied to mammalian cell imaging, immunoassays, and for testing the translocation mechanism of motor proteins in a "molecular car-crash".

P-583
DSC Characterization of protein aqueous solutions
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Calorimetric method and instrumentation were worked out and applied for investigation aqueous solutions of proteins. Thermal effects were analyzed by own construction heat-flux-type DSC cell designed for temperature range from the boiling point of water down to 120 K. The achieved sensitivity of heat flow rate (HF) of the instrument is better than 50☎W tested using 1 μl of 150 mM aqueous solution of NaCl. From the integral value of HF - the total enthalpy change ΔH_{total}, the enthalpies of transitions were separated from the heat capacities. Using the method, several types of proteins (BSA, ERD10, UBQ, α-, β-casein, and WT, A53T, α-synuclein mutants) were investigated in that temperature range. The results are shown in detail as an illustrative example. Potential applications are outlined, which include (*i*) the distinction between the solvent accessible surfaces of globular and intrinsically disordered proteins, (*ii*) the distinction between protein mutants, and (*iii*) the identification of monomer and polymer protein states. This method provides a possibility to study the polymerization process (amyloid formation) and to investigate *in-situ* the reason and circumstances of that.

P-584
Finite element approach to understand self gravitational bio in embryological compact mass
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Morphometry due to self gravity in living organism is an integral part in understanding *Self Gravitation Bio*. Computational studies of biological system, especially in ab-initio embryo as self gravitating mass, floating over amniotic fluid, as if maintaining anti – gravitation (extrinsic) mechanism, would be an interesting one to explore biomechanics of intrinsic gravity. Since the work would be of exploratory nature, both from the point of view of identifying appropriate stage of development of embryological morulla and available

computational logics, it was contemplated to initiate functional study with a few reported ultrasound evidential works on small animals like mice, grasshopper including compact human embryo as mathematical structure that may allow the formal definition of concepts such as convergence, mapping and continuity. As many of the finite-dimensional function analysis in topological vector spaces are available, we initiated our simulation and studies on concentrating locally compact banach spaces. On following differential equation in 3-D with requisite divergence, biparietal diameter and mentovertical diameter are parametrical values used to computationally simulate and model a final compact cranial topological mass.

P-586
Synthetic small molecules with cardiomyogenic effects on stem and progenitor cells
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Cell transplantation into the injured heart is a new therapy after myocardial infarction. Its success, however, is impeded by the limited capacity of donor cells to differentiate into functional cardiomyocytes in the heart. A strategy to overcome this problem would be the induction of cardiomyogenic function in cells prior to transplantation. Recently, synthetic small molecules (SySMs) were identified, which exhibit a remarkable capacity to trans-differentiate cells into other cell types. The aim of this study was to characterize SySMs with cardiomyogenic activity. Treatment of P19 embryonic carcinoma cells and C2C12 skeletal myoblasts with Cardiogenol-C (CgC) or MK142 significantly up-regulated cardiac marker expression. Thereby, CgC was especially active on lineage-committed C2C12, whereas MK142 on uncommitted P19 cells. Supporting this difference in target cell specificity, in studies on embryoid and cardiac body development from various cell types, CgC produced more beating cardiomyocytes from committed A5 cardiovascular progenitor cells, while MK142 more from uncommitted W4 mouse embryonic stem cells. We conclude that MK142 promotes mesodermal differentiation of stem cells, whereas CgC drives cardiomyocyte maturation of already induced mesoderm. Supported by AWS (Z090391).

P-587
Elements and relations of a Life Theory (LT)
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I propose a general theory about the genesis and nature of life(optimum). It seems to be based as a rule on interaction between symmetry and asymmetry ($\sim$ stability and flexibility) for equilibration. In that kind of multidisciplinary research these concept generalized and deepens previous trials to explain the question what is life.

Finally the data of this exploration show that nature of life is predictable by suitable methods for measurement like e.g. spectroscopy and probability.
As a result, the way of life goes the optimal friction by essential level of efficiency energy use.

O-589
Towards attosecond biophysics: what can ELI-ALPS offer?
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The Hungarian pillar of the ELI (Extreme Light Infrastructure) project, ELI-ALPS (Attosecond Light Pulse Source), is designed to open new vistas in attosecond science. It will be a high power, high repetition rate XUV/x-ray providing coherent pulses with attosecond and few-femtosecond time-structure allowing for practically all kinds of pump-probe experiments providing unprecedented access to the ultrafast electronic dynamics that constitutes the primary response of atomic and molecular systems to incident light. Especially important in understanding biomolecules is the combination of attosecond and few femtosecond pulses that will make possible the direct investigation of electron dynamics. Another key feature of ELI-ALPS that may find significant biological applications is its potential for sub-atomic 4D imaging. From a technical point of view it is important to emphasize that the high photon flux combined with extremely high (up to 100 kHz) repetition rate makes ELI-ALPS very user friendly in experiments where complex samples, such as biological material, are being studied.

O-590
Hydrodynamic synchronisation of light driven microscopic rotors
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Moving organelles of microscopic biological systems often tend to synchronize. It has been proposed that the origin of the synchronization is hydrodynamic interactions. However, there has been yet no direct experimental demonstration of the effect at the corresponding microscale. We used a functional model system to carry out experiments to demonstrate hydrodynamic synchronization.
We have produced micrometer sized propellers by two photon photopolymerization. They are held in optical tweezers and are rotated by light. Such rotors were held and rotated close to each other in optical traps produced by holographic optical tweezers. The distance of the rotors and the rotating torque could be freely varied. Synchronization of the rotation was demonstrated. The effect was characterized under a broad range of parameters. Based on the comprehensive data we built a model to describe the effect and tested it in detail.

Membrane structure

O-591
Single molecule biology—studying meetings within the plasma membrane

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In my lecture, I will show examples how to obtain insights into the organization of the cellular Nanocosm by single molecule experiments. Our primary goal is to understand the molecular organization of the plasma membrane and its impact on signaling processes. For this, we apply single molecule tracking to resolve the plasma membrane structure at sub-diffraction-limited length-scales by employing the high precision for localizing biomolecules of $\sim$15 nm. Brightness and single molecule colocalization analysis allows for studying stable or transient molecular associations *in vivo*. In particular, we developed a technique to detect molecular cluster formation directly in the live cell plasma membrane. With this methodology, individual aggregates can be selectively imaged, and the load of each cluster can be determined. We applied this technique to investigate the association of a fluorescent lipid analogues and lipid anchored proteins. Aggregates containing up to 4 probe lipids were observed to diffuse freely as stable platforms, shedding new light on the current debate concerning the existence of "lipid rafts". Using the same technique we could further quantify the subunit stoichiometry of ion channels. Finally, single molecule tools enable the direct observation of the random transitions a biomolecule experiences during its movements through the plasma membrane. For example, transient proximity due to interactions with other proteins can be directly monitored and quantified. I will present results on the interaction between antigen-loaded MHC and the T cell receptor directly in the interface region of a T cell with a mimicry of an antigen-presenting cell, using single molecule FRET.

O-592
The native M2 proton channel structure from influenza A

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Anfinsen's thermodynamic hypothesis states "that the native conformation (of a protein) is determined by the totality of inter-atomic interactions and hence by the amino acid sequence in a given environment." As in most Biochemistry textbooks, too often these last four words are ignored. For membrane proteins the complex and heterogeneous membrane environment can have a substantial impact on protein structure and dynamics as well as on our understanding of protein function. I will discuss a new structure of the M2 proton channel from Influenza A virus that is a proven drug target. With solid state NMR we have characterized the full conductance domain (residues 22-62) that has very similar electrophysiology to that of the full length protein. This structure has been characterized in a native-like liquid crystalline lipid bilayer environment. Additional structures have been achieved by x-ray crystallography as well as solution and solid state NMR, both with and without the antiviral drug, amantadine. Recently, resistance to the antiviral drugs amantadine and rimantadine has dominated the flu seasons and the recent swine flu pandemic. The resistance resulted from the S31N mutation in the proton conducting pathway. Considerable debate has arisen from the various structures of the M2 protein and their implications for both proton conductance and drug binding.
The solid state NMR conductance domain represents a highly constrained backbone structure with key sidechain restraints. In restrained Molecular Dynamics and QM/MM calculations a detailed model of the key HxxxW sequence responsible for acid activation, gating and conductance has been achieved at neutral pH. Based on this model it has been possible to develop a mechanism for proton conductance showing in detail how the charge on the histidine residues is stabilized and how protons are shuttled from the N-terminal pore to the C-terminal pore of this protein. The mechanistic details explain a broad range of chemical and physiological data.

P-593
Lipid composition is the dominant factor that determines the orientational states of membrane-bound antimicrobial peptides

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The perturbing activity of membrane-active peptides in cellular membranes is attributed to the protein-lipid interactions. A systematic correlation of peptide behavior with the bilayer properties should thus provide important clues about cytolytic mechanisms. Here, we have studied two structurally unrelated antimicrobial peptides, the cyclic β-stranded gramicidin S, and the α-helical PGLa. Using solid-state ^{19}F-NMR in oriented bilayers, we have previously characterized different alignment states for them in lipid membranes [*JACS* 130:16512; *Top.Curr.Chem.* 273:139]. On this basis, we can now use the signal of a specific ^{19}F-label as a fingerprint to monitor the re-alignment and mobility of these two peptides as a function of membrane composition. An NMR temperature series passing through the relevant lipid phase transition allows to address the state of the peptides in response to the lipid phase state. That way we have explored the effects of bilayer thickness, surface curvature, negative charge, and presence of cholesterol. These data are then compared with the peptide behaviour in plasma membranes from erythrocytes and bacterial protoplasts. We discuss the results with respect to the activities of these two peptides and evaluate the meaningfulness of the various membrane models.

O-596

Perforin induces invaginations in model membranes

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Perforin (PFN) is an important pore forming protein from the human immune system, which enables the clearing of virus-infected or tumor cells. PFN pores exist with a range of functional diameters. The larger pores allow the crossing of granzyme B across the membrane, but the direct passage of this probe appears to be rather slow process. We have also observed that PFN can induce the formation of invaginations and internal vesicles on giant unilamellar vesicles (GUVs) composed of pure lipids and when GUVs were formed from red blood cell ghosts. Invaginations formation was dependent on the presence of Ca^{2+} and functional PFN. The process of invaginations started rapidly and intraluminal vesicles were filled with fluorescent dextrans from the surrounding medium, irrespective of the dextran size. Intraluminal vesicles were complete and in some cases separated from the originating membrane. PFN was not uniformly distributed over the whole GUV surface but rather concentrated in the invaginations, according to the labeling by anti-PFN antibodies. The observed PFN activity may have implications for the transfer of granzymes into the cytosol of the target cells.

P-597

Phospholipid/cholesterol binary mixtures: polarity variations with composition and temperature

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Phosphatidylcholine (PC), sphingomyelin (SM) and cholesterol (Chol) are considered the major constituents of the outer lipidic leaflet of cell plasma membranes. The binary mixtures of PC and SM with cholesterol gives rise to significant differences in lipid bilayers properties, such as water permeability, thickness and molecular packing. Those variations will lead to some important changes in the fluidity and polarity gradients that can be measured within model membranes.

We studied the polarity variations of saturated (DPPC, egg-SM) and unsaturated (POPC, DOPC) phospholipids bilayers, and their respective mixtures with Chol at specific molar proportions, using the pyrene empirical polarity scale.

For POPC and DOPC the polarity values tend to decrease, either increasing cholesterol concentration, as well as temperature. For DPPC and egg-SM, we verified the same tendency for lower Chol contents. For higher proportions, and higher temperatures, we observe an increase in the equivalent polarity, which is much more pronounced for egg-SM.

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P-599

Hard X-ray phase contrast imaging of black lipid membranes

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We report on hard x-ray phase contrast imaging of black lipid membranes (BLMs), which are freely suspended over a micro machined aperture in an aqueous solution. This new way of membrane structure analysis allows investigating bio molecular and organic substances in aqueous environments by parallel and divergent beam propagation imaging, using partially coherent multi-keV x-ray radiation. The width of the thinning film is significantly smaller than the detector pixel size, but can be resolved from quantitative analysis of the intensity fringes in the Fresnel diffraction regime down to its native thickness of about 5nm. To our knowledge this is the first time that such small features of a very weak phase object have been visualized by direct x-ray imaging techniques. We have put forward a simplified but extendable model, which enables the theoretical description of image formation and characterization of membrane thickness and its decrease during the thinning process from a bulk to a bimolecular film. On the basis of recent experiments, future investigations will be performed to study the interactions of membranes, as they are for example known from synaptic fusion, with high spatial resolution.

P-600

Interaction between chorogenic acid and erythrocyte membrane

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Chlorogenic acid (CGA) is a compound of plant origin, being present in large quantities in coffee beans and fruits and leaves of apple tree. Scientific studies have documented a protective action of CGA on the human organism due to its antioxidant, anticancer, fungicidal and bactericidal properties. However, the effect of the acid on the molecular and cell levels have not yet been studied. The aim of the research presented was to determine the effect of CGA on the erythrocyte membrane and liposomes formed of membrane lipids. The fluorimetric method was used to determine the antioxidant activity of CGA, and its location within membrane on the basis of morphological changes of erythrocytes and fluorescence of some probes located in various regions of the membrane. The results indicate that CGA protects the red blood cell perfectly against oxidation. This research has

shown that the acid incorporates mainly in the exterior part of the erythrocyte membrane, inducing creation of echinocytes. This suggests that it interacts predominantly with the outer part of the lipid layer of erythrocytes and liposomes. It was also shown that the CGA decreases the packing order of the hydrophobic part of the membranes, without changing the anisotropic fluorescence of the hydrophobic part.
This work was sponsored by the Ministry of Science and Education, scientific project no. N N312 422340 and N N304 173840.

P-601
Giant unilamellar vesicle (GUV) as model system in advanced 3d orientation determination
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One of the unique features of single molecule absorption/emission is their anisotropy due to the well-defined transition dipoles for both processes allowing the determination of the molecule's 3d orientation. Therefore, several techniques have been proposed in order to determine the full 3d orientation of dipole emitters on a single molecule level. We recently demonstrated a technique that combines emission distribution and polarization detection [1,2,3]. As the method is an intensity distribution technique and based on single photon detection in principle, one can extend the 3d orientation determination to fluorescence correlation spectroscopy (FCS) as well as dynamical anisotropy measurements. This allows for the determination of the dynamics in 3d orientation of single molecules down to a nanosecond timescales. The 3d orientation is particularly interesting in non-isotropic environments. A lipid membrane is such a non-isotropic environment of enormous importance in biological systems. We therefore use giant unilamellar vesicle (GUV) labeled with dyes like DiO as a model system. Due to the defined curvature of such vesicles all possible dipole orientations can be achieved. This allows us to show the capabilities of our method on different timescales and to quantify the error in determination of 3d orientation dynamics in lipid membranes.

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P-603
Biological activity of strawberry leaf extract
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The aim of the studies was to determine the changes that occur in a biological membrane and the model lipid membrane as a result of interaction with strawberry leaf extract. Numerous studies conducted all over the world have documented a beneficial effect of polyphenolic compounds on the human organism. However, the mechanism of the interaction on the molecular and cell level is not yet known.
In the work presented, the effect of strawberry leaf extract on the erythrocyte and black lipid membranes has been investigated. The applied methods - spectroscopic, fluorimetric and electric - allowed to determine the hemolytic and antioxidant activity, and the packing order in the erythrocyte membrane as well as the electric capacity of BLMs.
The results obtained indicate that the extract is efficient in protecting membrane lipids against oxidation, does not induce hemolysis, increases osmotic resistance and decreases packing order in the hydrophilic region of the erythrocyte membrane. Moreover, it increases stability and life-time of flat lipid membranes, without altering their specific capacity.
The changes induced in erythrocyte membranes and BLMs indicate that strawberry leaf extract binds to the hydrophilic part of membrane.
This work was sponsored by the Ministry of Science and Education, scientific project no N N304 173840.

P-604
What is the difference between a supported and a free lipid bilayer? Insights from Molecular Modeling
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Supported Lipid Bilayers are an abundant research platform for understanding the behavior of cell membranes as they allow for additional mechanical stability and enable characterization techniques not reachable otherwise. However, in computer simulations these systems have been studied only rarely up to now. We present systematic studies on different length scales of the changes that a support inflicts on a phospholipid bilayer using molecular modeling. We characterize the density and pressure profiles as well as the density imbalance induced by the support. It turns out that the changes in pressure profile are strong enough that protein function should be impacted leading to a previously neglected mechanism of transmembrane protein malfunction in supported bilayers. We determine the diffusion coefficients and characterize the influence of corrugation of the support. We also measure the free energy of transfer of phospholipids between leaflets using the coarse-grained Martini model. It turns out that there is at equilibrium about a 2-3% higher density in the proximal leaflet. These results are in agreement with data obtained by very large scale modeling using a water free model where flip-flop can be observed directly. We are additionally characterizing the intermediate states which determine the barrier height and therefore the rate of translocation. We also study the influence of surface roughness and curvature on the behavior. Simulations in atomistic detail are performed for selected systems in order to confirm the findings.

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P-605

Lipid-soluble hydroquinone modifications induced on membranes

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We synthesized new alkylthiohydroquinones (ATHs); in order to investigate different aspects of lipid-soluble hydroquinones interactions with phospholipids normally found in cell membranes. They have the same long hydrophobic alky chains found in many lipids forming the cell membranes. In these compounds the tails should share the inner of the membrane, while the hydroquinone, as polar head group should remain on the surface. One or more alkylthio chains attached to the aromatic ring, modifies its hydrophobicity and should alter the electron distribution. Moreover, the two OH groups become chemically distinguishable, e.g. NMR spectroscopy and also show different pKa values.
We investigate the interaction of ATHs with lipid membranes, POPE and POPC and observe the formation of structures with different morphologies, or curvature, of the lipid membrane, depending on temperature and pH. We attributed their formation to changes in the balance charge/polarity induced by the ATHs. Mixtures with POPE at pH=4 forms two cubic phases P4332 and Im3m that reach a maximum lattice size while in basic conditions they only expand upon heating from room temperature. They coexist with lamellar or hexagonal and have been associated with inhomogeneous distribution of the ATHs molecules over the lipid matrix. The zwitterionic POPC does not form cubic phases, but instead shows two lamellar structures up to a high temperature.
In all mixtures of lipid/ATHs we observed the formation of lamellar and hexagonal phases, similar to the behaviour of pure hydrated lipid e.g. POPC, while for POPE additional cubic structures, depending on the environment conditions.

P-606

The role of the I-BAR domain in the filopodia formation

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The Inverse BAR (I-BAR) domain is part of the superfamily of the membrane-deforming protein is Bin-amphiphysin-Rvs (BAR) proteins which induce either positive or negative membrane curvature both *in vitro* and in cells. Generation of membrane curvature by these membrane deforming proteins often works together with actin dynamics. I-BAR shares its function between actin bundling and membrane binding but it is still obscured what molecular mechanisms are responsible for these functions. The aim of our project is to investigate the detailed membrane binding properties of the I-BAR of IRSp53 and its relations to the actin cytoskeleton. *In vitro* FRET experiments and fluorescence quenching studies were carried out between the I-BAR and liposomes made up from different lipid constructs. We have found that the I-BAR has preference to bind to the negatively charged lipids however it can also bind to the uncharged lipids. The fluorescence quenching studies reflected that the accessibility of the I-BAR surface was higher toward the negatively charged lipids than for the uncharged ones. TNS fluorescence assay reflected that the I-BAR domain binds to the surface of the micells rather than penetrating into its core.

P-607

Phase transitions and spatially-ordered counterion association in ionic-lipid bilayers: a statistical model

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Lipid bilayers present a well-known order-disorder chain transition at ambient temperatures. This transition may become anomalous if the lipid head-group presents ionic dissociation at low ionic strength, as detected by several experimental techniques: between the gel and the liquid phases an intermediate phase appears as a shoulder in the specific heat, a deep in turbulence or a maximum in conductivity. We propose a statistical model which allows ionic dissociation of the polar group on the membrane surface and thus introduces competition between the hydrophobic interaction of hydrocarbonic chains, which favours the gel phase, at low temperatures, and the electrostatic interaction of charged head-groups, which favours the fluid phase, at higher temperatures. The model presents an intermediate fluid phase with higher dissociation and charge ordering on the membrane surface, beyond a sharp gel-fluid transition. The model presents increasing temperature of the main transition by addition of salt, as well as the shrinking of the anomalous region as chain length increases. Model thermodynamic behavior is compared to results for PGs, phospholipids with a glycerol head-group.

P-609

Modulation of membrane fluidity by 2-hydroxylated fatty acid derivatives

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Keywords: Membrane fluidity, Minerval, 2-hydroxylated fatty acids.

Cellular functions are usually associated with the activity of proteins and nucleic acids. Recent studies have shown that lipids may alter the biophysical properties of lipid membranes and modulate the localization and activity of membrane proteins, thus regulation the cellular processes in which they are involved. The present work describes the effect of Minerval (2-hydroxyoleic acid), a potent antitumoral drug, and other different 2-hydroxylated fatty acid derivatives on the fluidity of lipid membranes. The self-quenching method of NBD-phosphatidylethanolamine showed that all the studied 2-hydroxylated fatty acids induced fluidification of domain-containing model membranes. However, phase separation was observed upon addition of these synthetic molecules on vesicles composed of homogeneously distributed lipids. Results of fluorescence confocal microscopy of giant unilamellar vesicles correlates with the disordering effects of the 2-hydroxylated fatty acids on the membrane structure. The ability of changing the membrane biophysical properties makes the 2-hydroxylated fatty acid derivatives candidates to be modulators of the membrane associated proteins activities and the subsequent intracellular cell signaling processes.

P-610

Transmembrane signalling through a heme/hemophore receptor, HasR

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The outer membrane (OM) of Gram-negative bacteria acts as a selective permeation barrier. The nutrients that cannot pass through porins are internalised *via* OM specific transporters by an active process depending on the energy given by an inner membrane complex. This is the case of iron sources, heme, vitamin B12, etc. We study on such an active transport system through an OM transporter, HasR. HasR is a central component of a heme acquisition system "Has" developed by several Gram-negative bacteria to satisfy their need of iron. It functions in synergy with a secreted heme carrier protein, named hemophore or HasA. HasA extracts heme from host hemoproteins as hemoglobin and then shuttles it to HasR, wherein it is internalized. The energy for HasR functioning is brought by the inner membrane HasB complex. The HasR OM receptor binds heme and HasA on its extracellular side and HasB on its periplasmic side. Using structural and biophysical approaches, we show that the binding of the substrate on the extracellular face of HasR modulates the interaction with HasB on the periplasmic face. The transmitted signal depends on the nature of the substrate[1,2,3,4].

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P-612

Target-selective membrane fusion with endosomal-pH-responsiveness

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The well-programmed membrane fusion systems, operating in a weakly acidic environment, have attracted attention in the fields of biochemistry, biophysics, and pharmacy because these acidic conditions are generally observed in endosomal membranes or tumor tissues. We have reported a selective liposomal membrane fusion system toward a sugar-like cyclic *cis*-diol structure on the target liposome. This system consists of a lapidated phenyl boronic acid derivative as membrane -bound fusogen and phosphatidylinositol as target. Here we report the preparation of a boronic acid / pH-responsive polypeptide conjugate as a novel membrane fusion device and the development of a target selective liposomal membrane fusion system with endosomal pH-responsiveness.
During the course of lipid-mixing, inner-leaflet lipid-mixing, and contents-mixing assays to characterize membrane fusion behavior, we clearly observed a liposomal membrane fusion phenomenon when the pH of the experimental system was changed from 7.4 (physiological) to 5.0 (endosomal). Our highly effective methods, which include a target selective liposomal membrane fusion, can be useful in the area of nanomedicine such as hybridoma technology and liposome-based drug or gene delivery.

P-613

Complete and reversible chemical denaturation of an α-helical membrane protein

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The question of how an unordered polypeptide chain assumes its native, biologically active conformation is one of the greatest challenges in molecular biophysics and cell biology. This is particularly true for membrane proteins. Chemical denaturants such as urea have been used successfully for *in vitro* un- and refolding studies of soluble proteins and β-barrel membrane proteins. In stark contrast with these two protein classes, *in vitro* unfolding of α-helical membrane proteins by urea is often irreversible, and alternative denaturation assays using the harsh detergent sodium dodecyl sulphate suffer from a lack of a common reference state.
Here we present the complete and reversible chemical denaturation of the bacterial α-helical membrane protein Mistic out of different micellar environments by urea. We applied multidimensional spectroscopy and techniques typically used in β-barrel membrane protein unfolding. Mistic unfolds reversibly following a two-state equilibrium that exhibits the same unfolded reference state. This allows for a direct comparison of the folding energetics in different membrane-mimetic systems and contributes to our understanding of how α-helical membrane proteins fold as compared with both β-barrel membrane proteins and water-soluble proteins.

P-614

Simple cell, complex envelope: modelling the heterogeneous membrane of *E. coli*

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Gram-negative bacteria such as *E. coli* are typically regarded as "simple" model organisms, yet their cell envelopes are surprisingly complex. Recent experimental and theoretical studies have revealed that contrary to the traditional view of the cell membrane as a passive bystander in membrane protein function, it plays a key role in protein folding, assembly, and function. To study the influence of the bacterial membrane on the dynamics of embedded proteins, we have performed atomistic molecular dynamics simulations of members of the TonB-dependent transporter family of proteins from *E. coli*. In particular, our aim has been to capture the details of lipid composition; we have modeled the outer membrane as an asymmetric bilayer containing an asymmetric distribution of lipopolysaccharide (LPS) and heterogeneous phospholipid mixtures. Our simulations enable us to explore the dynamics of the extracellular loops with a level of detail that is not possible using more traditional, simpler model membranes. By extending the simulation timescales out to hundreds of nanoseconds we are gaining new insights into the influence of "realistic" membrane environments on the structure and dynamics of membrane proteins.

P-615

Interaction between buckwheat extract and erythrocyte membrane

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In recent years, buckwheat has been of great interest in the world markets of healthy food, due to its high energy value, the content of unsaturated fatty acids, mineral constituents and vitamins. Its seeds contain flavonoids which are natural, efficient antioxidants.

The aim of the present studies was to investigate the effect of buckwheat extracts on the properties of biological membrane, which is the main site of the interaction between the substances buckwheat contains and the organism. The research was conducted on red blood cells and their isolated membranes, using the spectrophotometric, microscopic and fluorimetric methods. From the results obtained it follows that the compounds contained in buckwheat extracts increase the osmotic resistance of erythrocytes, making them less sensitive to the medium's osmotic pressure, induce changes in cell shape, producing increased number of echinocytes, and decrease the packing order of the polar heads of membrane lipids. It can thus be inferred that the compounds contained in the extracts penetrate the hydrophobic region of the erythrocyte membrane and alter its properties.

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P-616

Re-alignment of the antimicrobial peptide gramicidin S in lipid membranes: probing different sites of the molecule

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Gramicidin S (GS) is a cyclic decapeptide (*cyclo*[Val-Orn-Leu-^{D}Phe-Pro]).Due to its small size, symmetric structure, amphipathicity, proteolytic stability and testable mode of activity, the GS backbone is a convenient model system to examine the structure-activity relationship of individual amino acid substitutions.

We have previously reported the structure analysis of two GS analogues in which either the Val or Leu residues on the hydrophobic face of the molecule were substituted by the aliphatic ^{19}F-labeled amino acid 4F-Phg. Using ^{19}F-ssNMR in oriented lipid bilayers, we observed a re-alignment of the peptide that is compatible with the formation of a putative pore [*Top.Curr.Chem.* 273:139]. Here, we present novel analogs of GS with different ^{19}F-prolines in the β-turn region, and with CF_3-Bpg in place of Leu. Based on these ^{19}F-ssNMR results and supported by CD, DSC and activity tests, we could demonstrate that all analogues are structurally intact and antimicrobially active. We observe, however, differences in the re-alignment propensity when comparing these GS analogues in DLPC and DMPC bilayers. These differences can be rationalized in terms of molecular shape being changed upon incorporation of unnatural amino acids at various sites of the molecule.

P-618

Viral membrane fusion inhibited by beta-propiolactone treatment

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The beta-propiolactone (BPL) is an inactivating reagent commonly used to produce viral vaccine preparations (whole virions or split-virions). Although BPL has been reported to inactivate nucleic acids, its mechanism of action on proteins and the outcome on viral infection remains ill-defined. In this work, H3N2/Victoria/210/2009 influenza virus strain has been submitted to various BPL inactivation conditions (from 2μM to 1 mM). Cell infection ability was progressively reduced and entirely abolished at 1 mM BPL. To clarify the BPL effect, we focused on membrane fusion infection steps using kinetic fluorescence molecule leakage from liposome and lipid FRET assays combined with cryo electron microscopy. Membrane fusion measured at pH 5 on GM3 liposomes was reduced in a dose-dependent manner. Interestingly the fusion activity was

partially restored using the proton-ionophore monensin as confirmed by cryoEM images. In addition, a decrease of molecule leakage irrespective to BPL concentration was measured suggesting that the hemagglutinin affinity for GM3 was slightly modified even at low BPL concentration. Altogether these results strongly suggest that BPL treatment impairs M2 protein activity likely by preventing proton transport and bring new light on the mechanism of action of BPL.

P-619

Light scattering detects large vesicle deformations along the anomalous gel-fluid transition of DMPG

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The saturated anionic lipid dimyristoyl phosphatidylglycerol (DMPG), at low ionic strength, exhibits a very peculiar thermo-structural behavior. Along a wide gel-fluid transition region, DMPG dispersions display several anomalous characteristics, like low turbidity, high electrical conductivity and viscosity. Here, static and dynamic light scattering (SLS and DLS) were used to characterize DMPG vesicles. SLS and DLS yielded similar dimensions for vesicles in the gel and fluid phases. However, over the DMPG transition region, SLS indicated a threefold increase in vesicle radius of gyration, whereas the hydrodynamic radius, as obtained from DLS, increased by only 30%. Over the DMPG transition region, large positive values of the second virial coefficient clearly indicated an increase in inter-vesicle repulsion, probably due to the suggested increase in vesicle ionization. Despite the anomalous increase in the radius of gyration, DMPG lipid vesicles maintain isotropy, since no light depolarization was detected. Adding to results present in the literature, we propose an interpretation in terms of large fluctuations of vesicle form and bilayer density along the DMPG transition region, associated to head-group dissociation and formation of bilayer pores. Support: USP, FAPESP and CNPq.

P-620

Synergy between membrane-curvature and liquid ordered phase-state effectively sorts amphiphilic protein-anchoring motifs

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Cellular membranes have a heterogeneous lipid composition, potentially forming nano-domains or membrane rafts, believed to be platforms of altered fluidity involved in protein sorting and trafficking[1]. An alternative mechanism, potentially leading to protein sorting, has recently been proposed, suggesting that the curvature of membranes can also actively regulate protein localization[2]. Recently we showed that a variety of protein anchoring motifs are membrane-curvature sensors and thus up concentrate in regions of high membrane curvature[3]. Furthermore the curvature sensing ability of the anchoring motifs persisted independently of their structural characteristics. This leads us to speculate that curvature sensing might be an inherent property of any curved membrane and as a consequence, the lipid composition of the bilayer could potentially regulate this recruitment by membrane curvature. Thus there might be an intimate, yet unrecognized, link between the way raft-like membrane domains and membrane-curvature promotes the localization of membrane-anchored proteins.

We examined how changing the lipid composition of liposomes influenced the recruitment by membrane curvature of a model amphiphilic protein-anchoring motif. Employing our single liposome curvature assay, we tested lipid mixtures with different ratios of DOPC, sphingomyelin and cholesterol, giving rise to liposome populations of different phase-states. We found an amplified recruitment by membrane curvature for all raft-like l_o phase-state mixtures when compared to the l_d phase-state counterparts. Based on these findings we suggest a synergetic effect when combining a raft-like lipid phase-state and high membrane curvature, resulting in a highly potent mechanism for selective localization of membrane-anchored proteins.

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P-621

Minerval and other 2-hydroxylated fatty acid derivatives induce the formation of non-lamellar lipid structures

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Keywords: Non-lamellar lipid structure, phase transition, Minerval, 2-hydroxylated fatty acid.

Minerval (2-hydroxyoleic acid), a potent antitumoral drug, is known to modulate the lipid membrane structure by decreasing the lamellar-to-non-lamellar phase transition temperature (T_H). A series of 2-hydroxy fatty acid derivatives, varying in acyl chain length and degree of unsaturation, have been analyzed in terms of their ability to stabilize the inverted hexagonal (H_{II}) phase in palmitoyl-oleoyl-phosphatidylethanolamine membranes. Differential Scanning Calorimetry and ^{31}P-Nuclear Magnetic Resonance showed that mono- and polyunsaturated, but not saturated, 2-hydroxylated fatty acid molecules were able to decrease the T_H. Lipid vesicles mimicking the lipid composition of a cell membrane were solubilized at 4°C in the presence of Triton X-100. The results demonstrated that the amount of detergent-resistant membranes, which are related to liquid ordered (Lo) structures, decreased in the presence of 2-hydroxylated fatty acids. The so-called Lipid Membrane Therapy focuses on the reversion of cell disfunction through the modulation of the membrane structure, thus altering the

activity of membrane-associated proteins. The ability to modify the biophysical properties of a lipid membrane makes the studied 2-hydroxylated fatty acid molecules be prospective candidates for the use in the Lipid Membrane Therapy.

P-623

Steady-state and time-resolved fluorescence study of pyrenyl probes' excimer formation and quenching

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Keywords: Pyrene excimer, lateral diffusion, 2D steady-state kinetics, lipid bilayers

Excimer formation of phospholipid-labeled pyrenyl probes β-py-C_{10}-HPC and β-py-C_6-HPC in POPC MLV vesicles was examined by combined steady-state and time-resolved fluorescence. Our findings are in good agreement with the theoretical predictions by a kinetic formalism specific for fluorescence quenching processes occurring in two-dimensional media [Razi Naqvi *et al*. (2000) *J. Phys. Chem. B* **104**, 12035]. However, a significant downward divergence occurs above 4 mol % of probe content, which might indicate deviations to ideal mixing in fluid phase. Results for β-py-C_{10}-HPC, in mixtures of POPC with 10 and 20 mol % POPS were indistinguishable from those obtained with pure POPC vesicles; however excimer formation in pure POPS bilayers appears to be appreciably higher.
We also compared the excimer formation findings with quenching of the same probes by low concentrations of DOXYL quencher groups labeled acyl phospholipid chain at the same depth of the pyrenyl group. The results are also scrutinized by the same two-dimensional kinetic formalism and good correlation was also found.
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O-624

Biophysics of Membrane Lipidomics

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The amassing of comprehensive data on the lipid composition of biological membranes by lipidomics initiatives provides a potent challenge to the membrane biophysicist interested in lipid structure. This resolves itself essentially into two aspects. The first systematises the dependence of membrane biophysical parameters on lipid molecular structure. Lipid volumes, membrane dimensions, chain-melting temperatures and enthalpies, nonlamellar phase formation and structure, critical micelle concentrations and thermodynamics of membrane formation, membrane-membrane interactions and lipid transfer are amongst the properties of central biophysical interest. The relevant structural parameters are lipid chain length, degree of unsaturation, chain branching and headgroup configuration. The second, more complex and less well developed, aspect concerns the lipid-lipid interactions that determine the membrane properties of lipid mixtures. In part, these can be obtained from binary phase diagrams, and the more limited number of ternary phase diagrams - notably with cholesterol - that are available. Extrapolation to higher order mixtures lies in the future. I shall attempt to summarise some of the progress in these directions. The immediate aim is a second edition of my Handbook of Lipid Bilayers, which, in addition to a vastly expanded database, will include interpretative features and will be available in the early part of next year.

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P-625

Combined steady-state and time-resolved excimer formation analysis in POPC/cholesterol bilayers

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Lateral diffusion dynamics in phosphatidylcholine/cholesterol bilayers has been mostly accessed by means of EPR, NMR and FCS spectroscopic techniques. Reliable stady-state florescence quenching analysis of diffusion-controlled processes has been ampered by the lack of a self-consistent kinetic formalism for the two-dimensional (2D) counterpart of the classical Stern-Volmer analysis for three-dimensional (3D) solvents.
We studied the excimer formation of phospholipid-labeled pyrenyl probes (proportion of 4 mol %) in mixed POPC/Cholestrol MLV liposomes by combined steady-state and time-resolved fluorescence The findings are in very good agreement with the theoretical predictions of the kinetic formalism specific for fluorescence quenching processes occurring in 2D media [Razi Naqvi et al. (2000) J. Phys. Chem. B **104**, 12035]. We analyzed POPC/Cholestrol mixtures putatively in the liquid-ordered (35, 40 and 45 mol % Cholesterol) and in liquid-disordered (5 mol %) phases, and compare the outcomes with the results from pure POPC bilayers.
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P-626

The effects of 2N- and 3O-methylation of sphingosine on the membrane properties of ceramide in mixed bilayers

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The small hydrophobic head group, the closely packed acyl chains, and the capability of interfacial hydrogen bonding have been suggested to govern the characteristic membrane behavior of long chain saturated ceramides: the self-segregation, and the formation of hexagonal phases and highly ordered gel phases. While it has been shown that structural alterations of the ceramide acyl chains induce position dependent effects on their behavior, we wanted to study the effect of interfacial properties, including hydrogen bonding, on ceramide membrane properties. The H-bond donor functions of 2NH and 3OH in the sphingosine backbone of palmitoylceramide were disrupted either separately or simultaneously by replacing the hydrogen with a methyl-group. When the lateral phase behavior of mixed bilayers containing cholesterol/sphingomyelin-rich domains was studied in the presence of the ceramide analogs, the 3O-methylated ceramide appeared to form a thermally stable, sterol-excluding gel phase with sphingomyelin, whereas the 2O-methylated ceramide failed in both thermal stabilization and sterol displacement. The doubly methylated analog was the poorest ceramide mimic. Together with the possible steric effects induced by the methylations, the lack of 2NH H-bond donor function impaired ceramide membrane behavior to a greater degree than the lack of 3OH H-bond donor function.

P-628

Cytotoxicity and interactions of ω-(alkyldimethylamonium) alkylaldonamide bromides with membranes

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Sugar-based surfactants are made from renewable resources using the "green chemistry" methods, are easily biodegradable and used in washing agents, cosmetics, and drug carriers. Besides, there are attempts to use them as nonviral vectors in gene therapy. We studied the influence of new ω-(alkyldimethylamonium)alkylaldonamide bromides (C_nGAB) with different chain lengths (n = 10, 12, 14, 16) on the thermotropic phase behavior of DPPC, and DPPC/chol bilayers by means of differential scanning calorimetry. The surfactants were added either to the water phase or directly to the lipid phase (a mixed film was formed). We analyzed the changes in the temperatures, enthalpies and shapes of the main phase transitions as a function of concentration. Molecular modeling methods were also used. Cytotoxicicity of the C_nGABs was determined in the cell line L929 and A549. For cytotoxicity test, the cells were seeded in 96-well plates 1 ml of $2{\cdot}10^6$ cells/ml in the culture medium Eagle'a or Dulbecco with 2% calf serum, penicillin and streptomycin was deposited into each plates. The cells were treated with various doses of surfactants and incubated. The minimal concentration which was toxic to approximately 50% of cells was taken as $TCCD_{50}$. This work was supported by grant N N305 361739.

P-629

Direct visualization of large and protein-free hemifusion diaphragms

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Keywords: Membrane fusion, hemifusion, giant unilamellar vesicle, transmembrane domain

Membrane fusion is ubiquitous in life requiring remodeling of two phospholipid bilayers. As supported by many experimental results and theoretical analyses, merging of membranes seems to proceed via similar sequential intermediates. Contacting membranes form a stalk between the proximal leaflets which expand radially into a hemifusion diaphragm (HD) and subsequently open to a fusion pore. Direct experimental verification of the HD is difficult due to its transient nature. Using confocal fluorescence microscopy we have investigated the fusion of giant unilamellar vesicles (GUVs) containing fluorescent membrane protein anchors and fluorescent lipid analogues in the presence of divalent cations. Time resolved imaging revealed that fusion was preceded by displacement of peptides and lipid analogues from the GUV-GUV contact region being of several μm in size. A detailed analysis showed that this structure is consistent with the formation of an HD. A quantitative model of the hemifusion equilibrium and kinetics of the growing HD was developed. Bilayer tension could be shown to drive HD expansion and interleaflet tension was found to act as a counterforce, because the outer leaflets are compressed upon HD growth. The model and its predictions fit nicely with observations above.

P-630

Concentration effects of trehalose in the equivalent polarity of fluid POPC bilayers

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Trehalose is an important disaccharide, formed by two units of glucose linked by a α-1,1 glicosidic bond. It is capable of replacing water molecules in the hydration shell of the phospholipid headgroups, in cases of extreme dehydration, by establishing hydrogen bonds with their –CO and –PO groups, preserving this way the membrane structure. The polarity gradient is a significant feature of lipid bilayers and is influenced by the amounts of water within this medium. It is

therefore important to understand the effects of different concentrations of trehalose in simple model membranes.
Using the pyrene empirical polarity scale, we monitorized changes in the polarity values when varying trehalose concentration in the bounding aqueous phase. For lower concentrations (until 0.25 M), we observed a decrease in polarity, comparing with POPC bilayers in pure water. For higher trehalose concentrations (above 0.5 M), the polarity values are indistinguishable from those POPC in water.
Using the freeze and thaw technique we obtained the same results, except for the lower trehalose concentrations.
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O-631
Stereo-specific effect of the analgesic drug ketamine on lipid membranes
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General anesthetics are indispensible tools of daily surgery. Yet, their molecular mode of action remains elusive. While one school favors specific (direct) interactions with proteins of the central nervous system, another school adheres to a nonspecific modulation of biophysical membrane properties. One of the strongest arguments against lipid theories is the absence of stereo-specific effects in model membranes, as opposed to their detection by electrophysiological measurements on ion-channels. We have combined X-ray scattering and molecular dynamics simulations on palmitoyl-oleoyl-phosphatidylcholine bilayers with fluorescence microscopy on live cells to study the effects of the stereoisomers of ketamine on membrane properties. We find significant effects of both enantiomers on the distribution of lateral pressures at clinically relevant concentrations, being more pronounced for S-(+)-ketamine. We further calculated the effect of the lateral pressure profile changes on the opening probability of an ion-channel using crystallographic information. The observed channel inhibition compares remarkably well with clinically observed effects of the enantiomers. We thus provide first evidence for a stereo-specific, but indirect effect of general anesthetics on ion-channels.

O-632
Dependence of gramicidin A channel lifetime on membrane structure obtained from x-ray scattering measurements
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The activity of ion channels, in particular the lifetime of their conducting (open) state depends on the physical properties of lipid bilayers [1,2] which in turn depend on lipid headgroup and acyl chain composition. In order to investigate this dependence, we have performed measurements of gramicidin A (gA) channel lifetimes in three different lipid series. In each series, the lipid headgroups were phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS), while the acyl chains consisted of symmetric monounsaturated di(18:1), mixed (16:0)(18:1), and methylated di(16:0-4ME). In order to minimize the effect of headgroup electrostatics, measurements where performed in 1M KCl salts. We show how gA lifetimes depend on headgroup and acyl chain composition and on structural parameters determined by x-ray scattering. For the lipids considered, gA lifetimes cover a range from 0.7 seconds in the DOPE lipid to 18 seconds in DPhPS. In this range, we find a Gaussian dependence of gA lifetime on bilayer thickness, consistent with hydrophobic matching models. We discuss different aspects of channel-lipid interactions and to what extent measurements of gA lifetime in binary mixtures are consistent with measurements in pure lipid systems. [1] Rostovtseva *et al.*, Biophys. J. 94, L23-25, 2008. [2] Lundbaek *et al.*, PNAS, 107, 15427-15430, 2010.

P-633
Interaction between chlorogenic acid and model membranes
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The aim of the studies was to determine the effect of chlorogenic acid (CGA), which is the main constituent of plant extracts, on properties of the model membranes. Its effect was studied on temperature of the main phase transition of various lipids with and without presence of cholesterol, using the differential scanning calorimetry (DSC) method and the fluorimetric method. In particular, the degree of packing order of the hydrophilic phase of liposomes was determined using the laurdan and prodan probes, and fluorescence anisotropy of the hydrophobic phase with the probes DPH and TMA-DPH. It had also been studied the effect of chlorogenic acid on the structure and capacity of black lipid membranes (BLMs), formed of egg lecithin and lipids extracted from erythrocytes.
The results obtained indicate that CGA lowers the main phase transition temperature slightly, without changing the fluorescence anisotropy in the hydrophobic part of the bilyer, and causes a decease in the packing order of the hydrophilic phase. By monitoring the capacity during BLM formation we have found that the presence of chlorogenic acid accelerates the process of lipid self-organization into a bilayer, and increases stability and life of the BLMs. However, the was no effect of CGA on specific capacity of the membranes, and thus on thickness of the liposome membrane hydrophobic layer.
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P-634

Networking of sterols in lipid bilayers

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Many examples have recently been found where biological processes in the lipid bilayer are affected by the changes in the physicochemical properties of the membrane, e.g. the local curvature, the membrane tension and certainly the membrane structure. It has been shown that the activity of polyene antibiotics is strongly correlated to the phase diagram in a membrane composed of a mixture of POPC and ergosterol or cholesterol (*J Membrane Biol*, **237:**41-49, (2010)). It is known that polyene action is quite sensitive to the type of sterol in the membrane, which enables its medical use, mainly as antifungals. It has been proposed that this selectivity of the drug to fungi is related to structure modulation by the sterols (see for example, *Biophys. J.*, 85, 2323, (2003)) and therefore the correlation found could be due to structural differences between POPC/ergosterol and POPC/cholesterol along the corresponding phase diagrams. To investigate this, Molecular Dynamics simulations of the above mixtures along their phase diagrams were performed. It was found that there are indeed marked differences in structure along the phase diagrams, but for the sterol-sterol distribution function. An analysis of the behavior of this observable and the implications on polyene action is discussed.

P-635

Acyl transfer from lipids without enzyme catalysis: a new paradigm for membrane protein ageing?

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Membrane proteins are recycled *in cellulo* with half-lives ranging from minutes to days. In other systems, such as enveloped viruses, proteins may equally remain membrane-bound for periods of days. It is therefore of interest to examine the behaviour of proteins in model membranes over extended periods in order to determine the long-term stability of the mixed systems, both in kinetic terms (attainment of equilibrium states) and chemical terms (reactivity). The reactivity of proteins towards membranes has been examined using the peptide melittin as a model for membrane proteins. Acyl transfer from phospholipids to the peptide was found to occur over a period of several days, in the absence of any enzyme catalysis. Transfer was detectable after 2 days and reached 50% conversion in 8 days. Using tandem mass spectrometry approaches, the sites of melittin modification were localised. These sites included the side chain of lysine, opening the possibility that this residue may be modified in any membrane protein where this residue has an appropriate disposition. These observations challenge preconceptions concerning the membrane as an inert medium and highlight potential new mechanisms for membrane protein ageing.

P-636

Interaction of poly(L-arginine) with negatively charged bilayers studied by FT-IR spectroscopy

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Oligoarginine residues attached to macromolecules are known to facilitate the transport through lipid membranes. Since the mechanism of this transport is still unclear, the effect is often called “arginine magic”. We studied the interaction of poly(L-arginine) (PLA) of different molecular weight with negatively charged lipid bilayers. We have shown by calorimetric and monolayer techniques that the interaction is due to a combination of electrostatic and hydrophobic forces. Now we present an FT-IR spectroscopic study to reveal the effect of PLA binding on membrane organisation and peptide conformation. We will show that PLA binding reduces the lipid miscibility of negatively charged (PG or PA) and zwitterionic (PC) lipids within the bilayer. From the shift of the C=O stretching vibration we deduce that arginine side chains penetrate into the hydrophobic/hydrophilic interface and replace hydration water molecules. The binding reduces the rotational freedom of the lipid molecules, as could be shown by an analysis of the CH_2-streching vibrations. PLA binds in a β-sheet conformation to PG or PA gel phase membranes whereas its structure in bulk is random coil. The shift of the guanidyl vibration frequencies shows that also hydrogen bonds contribute to the PLA –lipid interactions.

P-637

Neutron scattering studies of model membrane as a function of hydration and temperature

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Cell membranes carry out highly specialised functions in living materials. The composition of bacterial membranes is essential to understand the mechanism of action of antimicrobial peptides. In order to understand the role of the various components contributing to the overall behaviour, we have reproduced the membrane of *Bacillus Subtilis* and carried out neutron diffraction studies on D16 (small momentum-transfer diffractometer) and D17 (reflectometer used as a diffractometer), at ILL. An ordered and homogeneous sample has been obtained by using the widely studied DMPC. The measured d-spacing of DMPC as a function of the relative humidity (RH) is related to the physical and chemical conditions affecting the sample. Consequently the reliability of the humidity chamber, which has been previously upgraded, has been stated. Moreover, the most suitable preparation technique has been set up.

In order to investigate the component roles within *Bacillus Subtilis* membrane, three samples of phospholipids were prepared (with POPE, POPG and cardiolipin). Neutron diffraction measurements, performed at controlled RH and temperature, suggested the presence of interesting phase transitions or coexistence of phases.

P-640

The rupture of membrane vesicles near solid surfaces

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The behavior of lipid membranes near solid surfaces has a great significance both in medicine and in technology. In spite of the widespread use and study of such membrane phenomena, their theoretical analysis is rather scarce. Our main goal here is to understand the process during which membrane vesicles first adhere to solid surfaces, then rupture (or go through a series of transient ruptures) due to the mechanical tension induced by the adhesion, and finally spread along the surface forming a supported lipid bilayer. In our theoretical description we simultaneously consider the dynamics of spontaneous pore opening and closing; volume loss via leakage through the pores; and the advancement of the adhesion front. All these processes are supposed to follow an overdamped dynamics and coupled to each other through membrane tension.
Our numerical simulations reveal that the rupture process consists of three well distinguishable phases: a fast initial volume loss; followed by a slow volume loss; ending with a final burst and surface spreading. The second phase can be skipped if either the first phase advances far enough or the third phase sets in early enough. The smaller the vesicle, the further the first phase can advance. The third phase can start earlier if either the surface is smooth enough, or the adhesion energy is large enough, or the line tension is small enough. When the second phase is not skipped the time needed for the rupture process can take very long with a large variance. In the realistic range of the material properties (line tension, bending rigidity) the process is qualitatively always the same, so the most decisive parameter remains the size of the vesicle: the smaller the vesicle the faster and easier it ruptures.

P-641

Liposomal delivery of antituberculotics

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Liposomes are applied to targeted drug delivery. Liposome-incorporated drugs have less side effects. Tuberculosis is caused by *Mycobacterium tuberculosis*. The bacteria can survive in macrophages. The aim was to develop liposomal drugs against TB. We made liposomes from diphalmitoil-phoshpatidylcholine (DPPC), or from dioleoyl-phosphatidyletanolamine (DOPE), cholesteryl hemisuccinate (CHEMS) and polyethilene-glycol releated distearoylphosphatidyl-ethanolamine (DSPE-PEG). We measured the size of liposomes with dynamic light scattering on the formulation day, than 2 and 7 days later. Samples were stored at 4 and 20 °C. We used isoniazid (INH) and peptide conjugated INHs as antituberculotics. We measured the drug encapsulation with fluorescence anisotropy. The liposomal drugs' in vitro activity was determined on *Mycobacterium tuberculosis* $H_{37}Rv$ culture and on *Mycobacterium tuberculosis* $H_{37}Rv$ infected MonoMac6 human monocyte culture. The DPPC liposomes showed significant aggregation both at 4 and 20 °C. Neither the empty, nor the INH-containing DOPE:-CHEMS:DSPE-PEG liposomes aggregated in 7 days. The INH and the peptide conjugated INH were encapsulated in both type of liposomes. Both liposomal drugs were effective in vitro on the bacterial culture and on the infected monocyte culture.

P-643

Direct observation of the effect of plant polyphenols on lipid domains in giant unilamellar vesicles

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Giant unilamellar vesicles (GUVs) produced by electroformation possess an average diameter of 30 - 50 μm and can be directly observed under fluorescence microscope if formed from a lipid-fluorescent label mixture. In the present study we used confocal microscopy technique to observe the morphology of lipid domains in GUVs prepared from ternary lipid mixture DOPC:cholestrol:sphingomyelin (1:1:1) and labeled with carbocyanine fluorescent probe ($DiIC_{12}(3)$). We chose four plant-derived polyphenols (flavonoids and stilbenes) of documented biological activity to study their influence on lipid domain number, area, shape, and border-length. We found that resveratrol elevated the number of domains per vesicle, decreased their area and markedly increased the total length of domain border without affecting domains' circular shape. Surprisingly, no such effect was observed for piceatannol differing from resveratrol by one hydroxyl group only. Neither genistein nor 8-prenylnaringenin changed the morphology of lipid domains significantly. The possible mechanism of resveratrol-induced effect on lipid domains' morphology could be its selective accumulation in the interfacial regions between liquid ordered and liquid disordered domains.

P-644

Putative cholesterol recognition amino acid consensus (CRAC) motif in HIV coreceptors CXCR4 and CCR5

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We identified a cholesterol recognition amino acid consensus (CRAC) motif in transmembrane domain 5 (TMD5) of

two G protein-coupled receptors (GPCRs), human chemokine receptors CXCR4 and CCR5, coreceptors of human immunodeficiency virus (HIV). We suggest that residues belonging to this CRAC motif are involved in cholesterol binding to CXCR4 and CCR5 that is responsible for cholesterol requirement for CXCR4 and CCR5 conformation and function and for the role that cell cholesterol plays in the cell entry of CXCR4-using and CCR5-using HIV strains. Putative CRAC sequences involve residues V^{214}/L^{216}-Y^{219}-K^{225} in CXCR4 and $L^{208}/V^{209}/V^{211}$-Y^{214}-K^{219} in CCR5. In CXCR4, CRAC motif is highly conserved across Chordata species, whereas in CCR5, CRAC motif is less conserved. Moreover, we identified CRAC motifs in TMD5 in 16 of 20 human chemokine receptors. In [Md. Jafurulla et al., Biochem. Biophys. Res. Commun. (2011), 404: 569] CRAC motifs in TMD5 of two other GPCRs were recently identified. CRAC motifs in these GPCRs, as putative CRAC motifs in CXCR4 and CCR5, include tyrosine residue that is highly conserved among GPCRs. This tyrosine is located close to the third intracellular loop, i. e., close to the membrane interface, as can be expected for an effective association with cholesterol.

Conformational dynamics, folding and IDP

O-645
Wide-line NMR and relaxation characterization of interfacial water in protein solutions

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^{1}H NMR FID, echoes, and relaxation times (T_1 and T_2) were measured at 82.55 MHz, at -70°C to +40°C, for lyophilized proteins, aqueous and buffered protein solutions. Examples for BSA, UBQ, ERD10, and WT α-synuclein and bird eye lenses are given. The melting of hydration water could be detected well below 0°C and the quantity of mobile water molecules (hydration, *h* vs. *T*) was measured. The *h* vs. *T* curves inform on the bonding character between the protein surfaces and H_2O. The magnitudes and the slope of the *h* vs. *T* curve describe quantitatively the interfacial landscape around the protein molecules and can be used for the distinction between the globular and IDP states. The behavior of the T_1 and T_2 data showed that there are two reorientation types present for every protein solutions below 0°C, irrespective for the nature of the protein or the solvent composition. Local field fluctuation and the BPP models were applied, which failed for the buffered protein solutions and for the IDPs dissolved in water. A main cause of the failure is the changing *h* in the analyzed temperature range. This case is valid for the solutions of IDPs and for buffered solutions of both protein types. Another cause can be the active relaxation channels other than dipolar when ions of quadrupolar nuclei are present.

P-646
Multinuclear NMR in buffered aqueous solutions of α-synuclein mutants

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^{1}H, ^{23}Na and ^{35}Cl nuclear magnetic resonance (NMR) free induction decay (FID) signals have been investigated in buffered wild-type (WT) and A30P α-synuclein protein solutions and in the solvent itself containing NaCl. The experiments have been carried out by Bruker SXP 100 and AVANCE III spectrometers at Larmor frequencies 82.1 MHz (^{1}H), 89.3 MHz (^{23}Na) and 33.1 MHz (^{35}Cl) in the temperature range of -150°C and 30°C. Below 0°C, the melting of hydration is followed by the broadening of the line width of all the three nuclei. The motional narrowing of the NMR spectra of the quadrupolar nuclei (^{23}Na, ^{35}Cl) is the consequence of the molecular motion of water molecules and the interaction of protein surface with sodium and chloride ions on a molecular level.

P-647
Calcium-induced structural, hydration and valence changes of intrinsically disordered RTX proteins

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Ligand-induced disorder-to-order transition plays a key role in the biological functions of many proteins that contain intrinsically disordered regions. This trait is exhibited by RTX (Repeat in ToXin) motifs found in more than 250 virulence factors secreted by Gram-negative pathogenic bacteria. We investigated several CyaA RTX polypeptides of different lengths ranging from 111 to 706 residues. We showed that the RTX proteins exhibit the hallmarks of intrinsically disordered proteins in the absence of calcium: they adopt premolten globule conformations and exhibit a strong time-averaged apparent hydration, due in part to the internal electrostatic repulsions between negatively charged residues, as revealed by the high mean net charge. Calcium binding triggers a strong reduction of the mean net charge, dehydration and compaction, folding and stabilization of secondary and tertiary structures of the RTX proteins. We propose that the intrinsically disordered character of the RTX proteins may facilitate the uptake and secretion of virulence factors through the bacterial secretion machinery. These results support the hypothesis that the folding reaction is achieved upon protein secretion and, in the case of proteins containing RTX motifs, could be finely regulated by the calcium gradient across bacterial cell wall.

P-648

C-terminal conformation changes of alpha-synuclein upon Al-induced oligomerization

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Occupational exposure to heavy metals has been recognized to be a risk factor for Parkinson's Disease via metal-triggered deposition of alpha-synuclein (αS)[1,2]. In the present work, Al^{3+} induced conformational change and instant oligomerization of αS have been studied using FRET and FCS as main techniques. Donor and acceptor were labeled in the C-terminal at positions A107C and A140C. The average lifetime of donor in the presence of acceptor increases with the increase of Al^{3+} concentration, indicating αS adopts a more extended conformation upon Al^{3+} binding. The intrinsic Tyr fluorescence rises sharply within the mixing dead time, reflecting an enhanced hydrophobicity of the Tyr environment and a fast conformational change of aS. Al^{3+} also induces an immediate oligomerization of αS as monitored by FCS. The diffusion coefficient of αS changes from 85 ± 5 $\mu m^2/s$ as monomer state to 23 ± 5 $\mu m^2/s$ as oligomer state. The oligomerization is supposed to be induced by the ligand bridging of trivalent Al ions.

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P-650

Using neutron scattering to investigate unfolded intermediates of ribonuclease A

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Ribonuclease A (RNase) is a well studied enzyme, catalyzing the degradation of RNA. As it provides a reversible transition following a distinct unfolding pathway, RNase A serves as a model system to investigate large scale thermal fluctuations inside the protein. This is one way to explore the energy landscape at a given equilibrium state and will show us possible rearrangements of biological relevant conformations as well as local stable conformations. By changing environmental parameters such as temperature, pH and solvent conditions, the level of energy can be varied.
We are investigating bovine pancreatic RNase A under different denaturation conditions using mainly small angle neutron scattering and neutron spin echo spectroscopy. Combining such techniques provides a unique tool to study the structural arrangement of proteins in solution and their dynamical properties. Large scale fluctuations of protein domains can be accessed as these techniques are sensitive to the structure and to collective dynamics. Using additional techniques, such as circular dichroism spectroscopy gives further insight into the secondary structure and how it responds to changes in temperature.

O-651

Fuzziness in protein-DNA interactions: beyond what can be seen

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Traditionally, specific DNA recognition is based on static contacts with the bases or phosphates. Residues far outside the binding context, however, can critically influence selectivity or binding affinity via transient, dynamic interactions with the DNA binding interface. The corresponding regions are usually intrinsically disordered (ID), which preserve their conformational freedom even when bound to DNA. There are four possible scenarios how distant ID segments impact DNA binding. They modulate *i*) conformational preferences, *ii*) flexibility or *iii*) spacing of the DNA binding motif(s) or *iv*) serve as competitive partners. Despite their low sequence similarity, the ID regions are often conserved at the structural level in families of orthologous proteins. Fuzzy protein-DNA complexes offer a variety of regulatory pathways via protein–protein interactions, post-translational modifications or alternative splicing in response to cellular cues.

P-652

Understanding the plasticity of c-Src tyrosine kinase through very long molecular dynamics simulations and experimentally validated free energy calculations.

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Nearly 2% of human genes encode protein-kinases (PK), enzymes involved in cellular signaling and several other vital biochemical functions, which transfer phosphate groups from ATP to specific target molecules, modifying their activity.[1] Deregulated PK have been linked to numerous diseases including cancer and diabetes, making them attractive targets for drug design.[2] Conformational transitions play a central role in regulating the phosphorylation activity. PK adopt an on state that is maximally active and one or more inactive states that shows minimal activity.[3] The similarity of the relatively rigid and largely conserved ATP binding site makes the design of selective inhibitors binding to the active state very difficult. Indeed some of the best cancer therapies available are based on inhibitors, as Imatinib, that bind to inactive states peculiar to a small subset of PK (Abl, c-Kit and PDGFR in the case on Imatinib). Thus, understanding the atomic details of the active to inactive transitions in kinases has a great importance. Here we study a particular active-to-inactive transition of c-Src, a fundamental proto-oncogene involved in cancer and metastasis, by using multi-microsecond long fully solvated molecular dynamics simulations, metadynamics and PTmetaD calculations [4,5]. The results, validated by mutagenesis, x-ray crystallography and binding kinetics, are suggestive of a functional role for the conformational transition. Moreover, we were able to single out the

most important residues affecting the conformational transition and to show that even a very conservative amino-acid substitution can have a dramatic effect on the conformational free energy landscape.

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O-653
Folding rates studied by a combination of static and time-resolved infrared spectroscopy
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The time-scales of protein folding events range over many orders of magnitude. In order to understand the complex folding mechanisms, peptides with well-defined secondary structure are often used as model systems as they may be regarded as smallest folding units of proteins. The formation of secondary structure elements occur on the nanosecond to low microsecond time scale. Thus, stopped-flow techniques are too slow whereas pulsed laser techniques are capable to trigger folding processes in nanoseconds and to analyze faster folding events. We study ns-to-μs peptide dynamics by temperature-jump infrared spectroscopy. After initiation of a nanosecond temperature jump, the spectral response is monitored at single wavelengths in the amide I region reflecting the dynamics of the peptide backbone. Relaxation rates are obtained. The helix-to-coil relaxation of polyglutamic acid is a multi-step process and requires more complex models than two-state kinetics. However, there are kinetic steps that are well described by single-exponential behavior and a two-state model. We demonstrate how equilibrium and time-resolved infrared spectroscopic data can be combined to deduce folding rates.

P-654
Protein unfolding and refolding by multidimensional spectroscopy
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Unfolding and refolding studies using chemical denaturants have contributed tremendously to our understanding of the thermodynamics and kinetics of protein folding and stability. However, a major limitation of this approach lies in the large uncertainty inherent in the extrapolation of the free energy of unfolding in the absence of denaturant from free energy values measured at finite denaturant concentrations.
Here we show that this limitation can be overcome by combining multiple spectroscopic signals—including fluorescence, circular dichroism, and absorbance—recorded in a quasi-simultaneous and fully automated way at different wavelengths. We have optimised the number of wavelength values used, the integration time per data point, the increment in the denaturant concentration, and the weighting scheme applied for global data fitting. Compared with the traditional approach based on the use of a single or a few wavelengths, we could thus improve the precision of the free energy value by an order of magnitude. We exemplify and validate this novel approach using representative, well-studied globular proteins and explain how it can be exploited to quantify subtle changes in membrane-protein stability which have thus far remained elusive.

P-655
Kinetic effect of Hofmeister ions on the photocycle of photoactive yellow protein
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Photoactive yellow protein (PYP) is a water-soluble photosensor protein in purple photosynthetic bacteria with a covalently bound p-coumaric acid chromophore. Light absorption initiates a photocycle with chromophore isomerization, transient protonation and conformational changes of various magnitude. The formation and decay of the consecutive photocycle intermediates of *Halorhodospira halophila* PYP after an actinic laser pulse were followed with time-resolved near UV – visible multichannel absorption spectroscopy in the 100 nanosecond – seconds range in buffers containing either NaF, NaCl or $NaClO_4$ in 0.66 M concentration at pH 8.2. Hofmeister salts – originally classified based on their salting-out or salting-in effects – influence the conformational stability of proteins. We have analyzed the spectrotemporal matrices obtained in different salts with the method Singular Value Decomposition with Exponential Fit Assisted Self-Modeling (SVD-EFASM[1]) and investigated the correlation of the Hofmeister salt-dependence of various rate constants of the PYP photocycle with the structural model of the protein's function.

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P-658
A model with two elastically coupled reaction coordinates for the internal friction of proteins
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The rates of protein conformational changes are usually not only limited by external but also internal friction, however, the origin and significance of this latter phenomenon is poorly understood. It is often found experimentally that a linear fit to the reciprocal of the reaction rate as a function of the viscosity of the external medium has a non-zero

value at zero viscosity, signifying the presence of internal friction. Furthermore, some of the experiments performed at different temperatures indicate that the internal friction follows an Arrhenius-like temperature dependence. To explain these phenomena we suggest a simple model for protein conformational changes in terms of two elastically coupled reaction coordinates, one of which being in contact with the external medium, and the other one experiencing the rough energy landscape of the protein. Our analytical calculations, supplemented with numerical simulations demonstrate that depending on the coupling strength (which is related to the flexibility of the protein) the short-wavelength components of the energy landscape roughness can be observed as an increased apparent internal friction with an apparent activation energy.

O-659

Probing cavities in SNase structure: a high pressure NMR study

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Since Bridgman seminal experiments on high-pressure denaturation of albumen in 1914[1], the physical basis of pressure unfolding is still largely unknown. We report here a specific study of cavities contributions to the volume difference between unfolded and folded states (ΔV_u), using four single point mutants of Staphylococcus nuclease (SNase). Each mutation is localised in a strategic position on the protein structure and was designed to change a large buried hydrophobic side chain into alanine, thus opening tunable cavities in the SNase 3D structure.
Measuring HSQCs peaks intensities up to 2500 bar monitored the equilibrium high pressure unfolding and leads us to precise estimations of ΔV_u for more than two-thirds of the 143 residues of each mutant. SO-FAST HMQC experiments[2] were also performed to measure folding and unfolding rates from 200 bar pressure jumps. High-pressure fluorescence experiments were done on six additional alanine mutants to complement the NMR study, allowing a more complete exploration of the local pressure sensitivity along the protein 3D structure. All these highly reliable measurements shed light on the real signification of the thermodynamic parameter ΔVu, and bring an unprecedented complex and heterogeneous picture at a residue level of the apparent two-state folding process of SNase.

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P-660

Differential hydration, void volume: which factor provides the main contribution to ΔV_u?

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Determination of contributing factors to the volume change magnitude between unfolded and folded states (ΔV_u) is a long-standing question in the high-pressure field. We provide here new experimental and computational data using two well-characterized model proteins: Notch ankyrin repeat domain (Nank) and Staphylococcal nuclease (SNase). The repetitive nature of the Nank protein was used to study influence of the protein size on ΔV_u in a systematic way with a set of deletion mutants. High-pressure fluorescence data provided new evidences that neither peptide bonds hydration nor side chains differential hydration could be considered as major contributor to the measured ΔV_u value. Additional molecular dynamics (MD) simulations rather suggested that the heterogeneous distribution of void volume in the folded states structures could explain the ΔV_u variations among the Nank deletion mutants. The specific issue of the void volume contribution to ΔV_u values was studied using 10 cavity mutants in SNase, allowing a large structural mapping of the alanine mutations on this globular protein. Combination of X-ray crystallography, high-pressure fluorescence, high-pressure NMR and MD simulations provided a first clear determination of the void volume contribution to the ΔV_u values. These results also bring an unprecedented complex and heterogeneous picture at a residue level of the apparent two-state folding process of SNase.

O-662

Lactoferrin: dynamics of a flexible protein in solution revealed by neutron scattering

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The understanding of the functionality of proteins started with a rigid model, namely the Lock and Key analogy, in 1894. Meanwhile, a more dynamic and flexible picture of these macromolecules has evolved to explain protein function like catalyzing biochemical reactions, transport, regulation, storage or defensive tasks. The importance of thermodynamically driven, internal motions for the functioning of proteins is subject of ongoing research.
We will present recent investigations of protein dynamics on Lactoferrin, a protein with antimicrobial activity which is part of the innate immune system. It consists of two binding sites,

each is capable of binding and releasing one iron ion. The crystallographic structures show that the binding sites have open and closed conformations, assumedly depending on the presence of iron (Andersen et al., Nature, 1990).
We are analyzing the internal dynamics of different binding states to elucidate of the binding mechanism with neutron scattering. Our unique method includes large scale structural characterization with small angle neutron scattering and the observation of internal motions of subdomains with neutron spin echo spectroscopy on nanosecond scale. This way we are able to clarify the link between binding mechanism and conformational change.

P-663
Comparison of the pressure- and temperature-dependent behavior of the ordered and disordered regions of titin
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Titin is a giant protein responsible for striated-muscle elasticity. It contains a series of ordered domains and a large disordered segment called the PEVK domain. The ordered domains belong to the immunoglobulin (Ig) type C2 and fibronectin (FN) type III superfamilies. The disordered PEVK domain is named after the residues proline, glutamate, valine and lysine which provide the majority of its constituents.
We expressed an Ig domain (I27) and a 170-residue-long fragment of the PEVK domain in order to investigate the effect of temperature and pressure on their conformation.
FTIR spectroscopy is a useful method for investigating the secondary structure of proteins. We analyzed the amide I band to obtain information on protein structure. Fluorescence labeling was also used in some experiments. To generate high pressures, a diamond anvil cell was employed.
The FTIR and fluorescence spectra of the protein fragments were recorded across the pressure and temperature ranges of 0-1 GPa and 0-100 °C, respectively. Moderate changes were observed in the conformation of the PEVK fragments in the explored range of the *T-p* plane, suggesting that the domain is a highly flexible, random-coil across the entire studied *T-p* range. By contrast, the I27 domain showed quite stable secondary structure.

P-664
Impact of variable DMSO additives on the catalytic and calorimetric performance of α-chymotrypsin
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New insights in understanding of fundamental links between proteins' stability, conformational flexibility and function can be gained through altering protein's thermodynamic and dynamic properties. In the present work an impact of non-specific moderate denaturant, dimethylsulphoxide, DMSO, on the kinetic (functional) and thermodynamic (thermal stability) patterns of a hydrolytic enzyme, α-chymotrypsin (α-CT) has been investigated. For the α-CT-catalysed process of ATEE hydrolysis, within the DMSO concentration range of 0-25% V/V we observed increase of the Michaelis constant (K_M) whereas the catalytic constant (k_{cat}) remained unchanged. These observations indicate on a preservation of the protein's active conformation accompanied by increase of the conformational flexibility of its active centre. In the presence of similar DMSO concentrations microcalorimetric data reflecting the α-CT thermal denaturation clearly point to the stability increase of the protein's compact part. Subsequent increase of the DMSO concentration leads to the protein's global destabilization, however the enzymatic hydrolysis process still obeys the Michaelis-Menten pattern, indicating about the involvement of the molten-globule-like state of α-CT.

O-665
How scarce sequence elements control the function of single ß-thymosin/WH2 domains in actin assembly
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β-thymosin (ßT) and WH2 domains are widespread, intrinsically disordered, proteins that fold upon binding actin. They display significant sequence variability associated to versatile regulations of actin assembly in motile processes. Here we reveal the structural basis by which, in their basic 1:1 stoichiometric complexes with actin, they either inhibit assembly by sequestering actin monomers (Thymosin-ß4), or enhance motility by directing polarized filament assembly (Ciboulot ßT or WASP/WAVE WH2 domains).
We combined mutational, functional, and structural analysis by X-ray crystallography, SAXS and NMR on Thymosin-ß4, Ciboulot and the WH2 domain of WASP-interacting protein (WIP). Functionally different ßT/WH2 domains do not target alternative actin binding sites but rather differ by alternative dynamics of their C-terminal half interactions with G-actin pointed face. The interaction dynamics largely depends on the strength of electrostatic interactions of a single residue along their sequence. The results open perspectives for elucidating the functions of ßT/WH2 domains in other modular proteins and enlighten how intrinsic structural disorder can lead to a novel mode of functional versatility.

P-666
Interfacial water in β-casein molecular surfaces: wide-line NMR, relaxation and DSC characterization
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Wide-line proton NMR FID, echoes, spin-lattice and spin-spin relaxation times were measured at 82.55 MHz frequency in the -70°C to +40°C temperature range, in lyophilized β-casein and aqueous and buffered solutions, and DSC method were also applied. The motivation for the selection of β-casein is the uncertainty of structural order/disorder. Naturally, the NMR and thermal characteristics were also evaluated. The melting of hydration water could be detected well below 0°C and the quantity of mobile water molecules (hydration) was measured. The hydration vs. melting temperature curve has informed us on the bonding character between the protein surfaces and water molecules. The generally used local field fluctuation model and the BPP theory were applied in the interpretation, and the limits of the models were concluded.

O-667
Promiscuous liaisons: functional interactions of intrinsically disordered proteins in biological signaling
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Intrinsically disordered proteins participate in important regulatory functions in the cell, including regulation of transcription, translation, the cell cycle, and numerous signal transduction events. Disordered proteins often undergo coupled folding and binding transitions upon interaction with their cellular targets. The lack of stable globular structure can confer numerous functional advantages, including, paradoxically, both binding promiscuity and high specificity in target interactions. NMR is unique in being able to provide detailed insights into the intrinsic conformational preferences and dynamics of unfolded and partly folded proteins, and into the mechanism of coupled folding and binding. The function of intrinsically disordered protein domains in transcriptional regulation and signaling will be described, with particular reference to the general transcriptional coactivators CBP and p300, the tumor suppressor p53, and the adenovirus E1A oncoprotein. The globular domains of CBP/p300 are targets for coupled folding and binding of disordered transactivation motifs of numerous transcription factors and viral oncogenes, which compete for binding to limiting amounts of CBP/p300. Many intrinsically disordered proteins contain multipartite interaction motifs that perform an essential function in the integration of complex signaling networks. The role of multipartite binding motifs and post translational modifications in regulation of p53-mediated signaling pathways will be discussed.

Systemic and collective behavioural aspects in biology

O-668
Vasculogenesis and collective movement of endothelial cells
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The early vascular network is one of the simplest functioning organs in the embryo. Its formation involves only one cell type and it can be readily observed and manipulated in avian embryos or in vitro explants. The early vascular network of warm-blooded vertebrates self-organizes by the collective motility of cell streams, or multicellular "sprouts". The elongation of these future vascular network segments depends on a continuous supply of cells, moving along the sprout towards its tip. To understand the observed self-organization process, we investigate computational models containing interactions between adherent, polarized and self-propelled cells. By comparing the simulations with data from in vivo or simplistic in vitro experiments, we explore the role of active migration, leader cells, invasion of the ECM, and cell guidance by micromechanical properties of adjacent cell surfaces.

P-669
Isotopic dilution mass spectrometric transmembranar transport study of amino acids in human erythrocytes
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A study of amino acid transmembranar transport in human red cells is presented. An isotopic dilution GC/MS technique was developed for in vitro transport study of leucine and glycine in human erythrocytes. ^{15}N-labeled glycine and ^{15}N-leucine were used as internal standards. The amino acids values were calculated by using two different methods, by using regression curves or the least squares method. The methods were validated and good values were obtained for parameters as linearity, precision and accuracy. Small values for both amino acids transport were measured. Influences of parameters such as time, temperature and electromagnetic field on transmembranar transport of amino acids were studied.

P-670
Logic estimation of the optimum source neutron energy for BNCT of brain tumors
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Boron Neutron Capture Therapy (BNCT) is a promising method for treating the highly fatal brain tumor; glioblastoma

multiform. It is a binary modality; in which use is made of two components simultaneously; viz. thermal neutrons and Boron-10.
The biophysics of BNCT is very complicated; primarily due to the complexity of element composition of the brain. Moreover; numerous components contributes to the over all radiation dose both to normal brain and to tumor. Simple algebraic summation cannot be applied to these dose components, since each component should at first be weighed by its relative biological effectiveness (RBE) value. Unfortunately, there is no worldwide agreement on these RBE values.
Thermal neutrons were formerly employed for BNCT, but they failed to prove therapeutic efficacy. Later on; epithermal neutrons were suggested proposing that they would be enough thermalized while transporting in the brain tissues. However; debate aroused regarding the optimum source neutrons energy for treating brain tumors located at different depths in brain. Insufficient knowledge regarding the RBE values of different BNCT dose components was a major obstacle.
A new concept was adopted for estimating the optimum source neutrons energy appropriate for different circumstances of BNCT. Four postulations on the optimum source neutrons energy were worked out, almost entirely independent of the RBE values of the different dose components. Four corresponding condition on the optimum source neutrons energy were deduced. An energy escalation study was carried out investigating 65 different source neutron energies, between 0.01 eV and 13.2 MeV. MCNP4B Monte_Carlo neutron transport code was utilized to study the behavior of these neutrons in the brain. The deduced four conditions were applied to the results. A source neutron energy range of few electron volts (eV) to about 30 keV was estimated to be optimum for BNCT of brain tumors located at different depths in brain.

P-671
Simulation of mutation induction by inhaled radon progenies in the bronchial epithelium
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Radon is considered as the second most important cause of lung cancer after smoking. To understand the mechanisms leading from radon exposure to cancer formation is of crucial importance. This study focuses on the description of mutation induction by radon progenies in the bronchial epithelium. Computational fluid and particle dynamics approach was applied to determine the radio-aerosol deposition distribution in the central airways. A numerical replica of a small fragment of the bronchial epithelium was prepared based on experimental data. Microdosimetric computations were performed to quantify the cellular radiation burdens at the very site of deposition accumulation. A mutagenesis model was applied supposing that radiation induces DNA damages and enhances the cell turnover rate.
The results show that both considered mutagenic effects of densely ionising radiation contribute significantly to mutation induction and mutation rate depends non-linearly on exposure rate. Furthermore, simulations suggest that the local maintenance capacity of the bronchial epithelium can be exhausted by chronic exposure to radon progenies with activity concentration characteristic of some uranium mines. The present work demonstrates possible applications of numerical modelling in radon related carcinogenesis studies.

P-672
On Future approaches to biophysics
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Introduction: Globalization needs new organizational models also for biophysics. Reports on necessity of int. institutes for biophysics (IIB) c/o int. universities (proposed by British Nobel Laureate B.Russell) are given (Neu et al. Basic&Clin. Pharm.&Tox. 17/S1,489-2010; J.Physiol.Sci. 59/S1,447/8-2009; Eur.Biophys.J. 34/6,819-2005).
Conception: Proposals for **EBSA**-discussion: *1.* Enlargement of Executive Committee by *a. honorary & presidents* (permanent 1-3: moral support & 1-3 fixed term), *b. Interdisciplinary commission:* Scientists from biology, medicine, physics, etc. (FEPS/IUPS, IUPHAR, IUPAB, etc). *2.* Implication of *interdisciplinary topics* to ESBA/IUPAB *congress-programmes, 3.* also for biophysical journals. *4.* Organization of *common interdisciplinary sessions* not only to biophysical, but also to other congresses. *5.* Co-operation between ESBA/IUPAB with int. interdisciplinary organisations (WAAS, ICSD/IAS, Eur. academies) for creation of *IIB* by network of national ones: Successive common personnel, possibility for whole life work, etc.
Conclusion: Realization of proposals 1.-5. could increase scientific/political authority of EBSA/IUPAB, leading to model for renewal of scientific organizations, supporting **UNO-Agenda21** for *better health, education, ecology, economy in all countries*.

O-673
Collective migration of neural crest cells: a balance between repulsion and attraction
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The Neural Crest is a group of cells found in all vertebrate embryos. It forms in the neural folds at the border of the neural plate and gives rise to a huge variety of cells, tissues and organs. One of the astonishing characteristic of neural crest cells is that they are able to migrate very long distances in the embryo. The neural crest has been called the “explorer of the embryo” as it is one of the embryonic cell types that migrate most during development, eventually colonizing almost every tissue.
In this talk I will discuss our recent finding about neural crest migration. We have shown that neural crest cells, classically described as mesenchymal cells, migrate in large clusters

and that interactions with neighbor cells are essential to control directional migration. One of these interactions is contact inhibition of locomotion [Nature. 2008. 456, 957] and I will show evidence of a new cell interaction behavior that we have recently discovered. I will present our cellular and molecular data that identify collective cell migration as a feature of neural crest and the molecules responsible for such behavior [Dev Cell. 2010. 19, 39]. Finally, I will discuss some mathematical modeling that integrates different kinds of cell interactions to explain collective neural crest migration, and tumor metastasis.

O-674

Pattern formation by collective cell migration-driven segregation

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Pattern formation by cell sorting is an important process in various biological events including embryonic development. To investigate the impact of collective cell migration on cell sorting we used fluorescent time-lapse videomicroscopy in two-dimensional mixed cell cultures made of various pairs of cell types with different motility characteristics. We demonstrate that more correlated cell migration, characterized by longer directional persistence length of migrating cells, results in more extensive segregation into homogeneous cell clusters, monitored by various statistical physical methods.

O-675

Signaling cascade dynamics after a hyper-osmotic shock in the yeast *Saccharomyces cerevisiae*

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Eukaryotic cells use signaling pathways to detect stimuli from their environment and convey this information to the nucleus where transcriptional adaptation takes place. Mitogen Activated Protein Kinases (MAPK) pathways are involved in several cellular responses such as stress reaction or proliferation controlFADDIN EN.CITE . The High-Osmolarity Glycerol (HOG) pathway in the yeast *Saccharomyces cerevisiae* is in charge of the osmo-regulation and its activation leads to the nuclear translocation of the protein Hog1p. The dynamics of this cascade to mild osmotic shock has been recently investigated by several groups both experimentally and theoretically. Here, we combine a microfludic device with fluorescent microscopy to study the HOG signal transduction dynamics in response to increasing osmotic shock. We show that the nuclear translocation dynamics of Hog1p is slowed down when increasing the osmotic shock intensity. Surprisingly, the cascade is still operational, since Hog1p phosphorylation is not altered. Moreover, we show that this slow down is universal since other signaling pathways are also severely slowed down when activated in a hyper osmotic environment. Our results suggest that the nucleocytoplasmic transport is altered by hyper osmotic shocks.

O-676

Polar actin cortex mechanics and cell shape stability during cytokinesis

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Cytokinesis relies on tight regulation of the mechanical properties of the cell cortex, a thin acto-myosin network lying under the plasma membrane. Although most studies of cytokinetic mechanics focus on force generation at the equatorial acto-myosin ring, a contractile cortex remains at the poles of dividing cells throughout cytokinesis. Whether polar forces influence cytokinetic cell shape is poorly understood. Combining cell biology and biophysics, we demonstrate that the polar cortex makes cytokinesis inherently unstable and that any imbalance in contractile forces between the poles compromises furrow positioning. We show that limited asymmetric polar contractions occur during normal cytokinesis, and that perturbing the polar cortex leads to cell shape oscillations and division failure. A theoretical model based on a competition between cortex turnover and contraction dynamics accurately accounts for the oscillations. We further propose that blebs, membrane protrusions that commonly form at the poles of dividing cells, stabilise the position of the cleavage furrow by acting as valves releasing cortical contractility. Taken together, our findings show that the physical properties of the entire cell are integrated into a fine-tuned mechanical system ensuring successful cytokinesis.

O-677

Transition to collective motion in bacterial colonies

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Collective motion of individual cells marks the onset of the transition to multicellularity in many microorganisms. This transition is often mediated by intercellular communication signals between cells. Here, we show, in contrast, that the transition from single cell to collective motion in an ensemble of gliding bacterial cells can be understood as a dynamical self-assembly process of self-propelled rods. Experiments were carried out with a mutant of the bacterium Myxococcus xanthus moving by means of the A-motility system only and without undergoing reversals. The collective motion phase is confined to a monolayer and is characterized by the organization of cells into larger moving clusters. A transition to collective motion is detected in experiments by image analysis, that reveals a qualitative change of the cluster-size distribution at a critical cell packing fraction around 17%. This transition is characterized by a scale-free power-law cluster size

distribution with an exponent 0.88. We provide a theoretical model for cluster formation of self-propelled rods that reproduces the experimental findings for the cluster size distribution. Our findings suggest that the interplay of self-propulsion of bacteria and volume exclusion effects of the rod-shaped cell bodies is sufficient to explain the onset of collective motion and the related changes in the cluster statistics.

Single molecule biophysics

O-680
Millisecond-piconewton force steps reveal the kinetics of DNA overstretching

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Until now the kinetics and energetics of the transition from the basic conformation of ds-DNA (B state) to the 1.7 times longer and partially unwound conformation (S state) have not been defined. The force-extension relation of the ds-DNA of λ-phage is measured here with unprecedented resolution using a dual laser optical tweezers that can impose millisecond force steps of 0.5- 2 pN via bead attached to opposite strands of the molecule (temperature 25 °C). This approach reveals the transition kinetics of ds-DNA elongation and their load-dependence. We show that (1) the elongation (ΔL) following the force step imposed on the molecule in the region of overstretching transition has an exponential time course with a rate constant (r) that has a U-shaped dependency on the force attained by the step (F); (2) the r-F relation is unaffected by the reduction of the force step size from 2 to 0.5 pN; r is related to the extent of elongation ΔL_0 through a power equation that, once linearized by double log transformation shows a slope of $\sim$0.6. These results are interpreted with a two-state reaction model that provides structural information on the transition state and an estimate of the size of the elementary reaction step of 25 bp. Supported by Ente Cassa di Risparmio di Firenze.

P-681
Single molecule microscopy: from nanodiamonds to nanomanipulation

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Single molecule based techniques made their way from studies of biological dynamics and conformations, towards DNA sequencing and ultra high resolution imaging. From the very beginning, confocal microscopy was the workhorse due to its versatility and straight forward multiparameter detection capability.

Photon coincidence measurements (antibunching) started as the ultimate proof for the existence of a single emitter but nowadays also decipher the true number of immobilised emitters in the sub-micron observation volume. We use this technique to characterise a new and promising class a luminescent labels, NV defect centers in single nanodiamonds, where knowledge about the number of independent emitters in a single nanodiamond is the prerequisite to understand the complex fluorescence decay behaviour.

The combination of atomic force microscopy (AFM) with single-molecule-sensitive confocal fluorescence microscopy enables a fascinating insight into the structure, dynamics and interactions of single biomolecules and their assemblies. Sub ensemble single fluorophore counting becomes possible as well as the observation of sub-diffraction imaging features. Adding an AFM tip to the confocal observation volume allows to complement the optically acquired information with topographic imaging. In addition, nanophotonic effects, such as fluorescence quenching or enhancement due to the AFM tip, are used to increase the optical resolution beyond the diffraction limit, thus allowing to identify different fluorescence labels within e.g. a macromolecular complex. Silicon tip induced single molecule quenching could be demonstrated on individual organic fluorophores [1].

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P-682
Simulation of chain extension of folded and unfolded DNA molecules in nanochannels

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Molecular simulations combined with the concepts of polymer physics expand our understanding of single molecule experiments. Stretching and confining in nanoscale channels and capillaries is used e.g. for sorting fragments of dsDNA of different length. We have employed Monte Carlo simulations based on a discrete worm-like chain model to compute the chain extension R of DNA as a function of channel dimension D (*J. Phys. Chem. B*, 2009,113,1843). The chain extension profiles $R(D)$ computed for a rectangular channel, tube and slit displayed three sections, in qualitative agreement with nanofluidic measurements of DNA at high salt concentrations. DNA behavior in channels of intermediate widths was explained by the statistics of ideal chain blobs and the scaling relation with the exponent of about x=-1. The transition to regime in narrow channels occurs when the diameter D is about equal to the DNA persistence length. Simulations revealed a considerable amount of folded structures of DNA molecules. Since hairpins are much shorter than the straight forms, they tend to

significantly reduce the equilibrium chain extension *R*. The back-folding of DNA chains is manifested especially at intermediate channel widths. The individual folding-unfolding events can be identified in MC traces.

P-683
NanoTracker: force-sensing optical tweezers for quantitative single-molecule nanomanipulation
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In the past decade, experiments involving the manipulation and observation of nanostructures with light using optical tweezers methodology have developed from proof-of-principle experiments to an established quantitative technique in fields ranging from (bio)physics to cell biology. With optical tweezers, microscopically small objects can be held and manipulated. At the same time, the forces exerted on the trapped objects can be accurately measured. JPK has developed a quantitative optical tweezers platform, the NanoTracker. This platform allows the controlled trapping and accurate tracking of nanoparticles, suspended either in a microfluidic multichannel flow chamber or even in a temperature-controlled open Petri dish. With its 3D detection system, particle displacements in the trap can be recorded with nanometer precision. Moreover, dynamic forces acting on the particle (e.g. exerted by motor proteins) can be measured with more than piconewton resolution on a sub-millisecond time-scale. Several successful biophysical applications will be demonstrated. In particular, we show how one of the hallmarks of single-molecule biophysics, the overstretching transition of DNA, can be studied in a versatile manner and used for protein-DNA interaction mechanics.

O-684
Magnetic tweezers studies of AddAB: a molecular motor for repairing broken DNA
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In *Bacillus subtilis*, broken DNA ends are first processed to a 3′-ssDNA overhang terminated at a recombination hotspot (Chi) sequence. This reaction is catalysed by the AddAB helicase-nuclease that unwinds the DNA duplex and degrades the nascent single-strands in a Chi-regulated manner (Yeeles and Dillingham, 2007). We have recently shown that recombinational hotspots regulate AddAB function by preventing reannealing of nascent single strands via formation of a DNA loop (Yeeles et al., 2011). Here, we have used Magnetic Tweezers to measure the real-time dynamics of AddAB at the single molecule level. We have measured AddAB translocation rate for Chi-containing and Chi-free DNA molecules which showed a complex appearance with the presence of constant-velocity segments of 300 bp/s at RT punctuated by stochastic pauses. Pauses duration followed a single exponential distribution with a decay time of 0.6 s. Interestingly, specific nicking in the AddAB translocating strand in both Chi-containing and Chi-free substrates favored pausing of AddAB at this nicked position, but did not prevent further AddAB translocation. Moreover, we observed that the presence of Chi sequences increases the frequency of AddAB pausing and reduces the AddAB translocation rate. The molecular basis for these observations is currently under evaluation.

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Yeeles, J. T., van Aelst, K., Dillingham, M. S., and Moreno-Herrero, F. (2011). Recombination hotspots and single-stranded DNA binding proteins couple DNA translocation to DNA unwinding by the AddAB helicase-nuclease. Molecular Cell (In press).

O-685
Direct observation of the interconversion of normal and pathogenic forms of α-synuclein
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Despite much speculation on the existence of structurally distinct oligomeric species associated with the conversion of certain monomeric proteins into amyloid fibrils, it has not previously been possible to observe them directly or to relate them to any key mechanistic steps involved in the interconversion process. We have developed a novel application of single-molecule intermolecular FRET to investigate in unprecedented detail the aggregation and disaggregation of alpha-synuclein, the protein whose pathogenic deposition as intracellular Lewy bodies is a characteristic feature of Parkinson's disease. Our study reveals that a range of oligomers of different size and structure are formed, even at physiologically relevant concentrations. Interestingly, the resistance to degradation of the aggregated state of alpha-synuclein, which is a well-

established generic characteristic of amyloid fibrils, results from a specific conformational transition that we have been able to observe directly. Moreover, we show that this transition converts disordered into ordered soluble oligomers and is remarkably slow, and indeed is likely to be the molecular origin of the disruption to the normal clearance mechanisms of the cell that is associated with protein deposition.

O-686
Single-molecule torque spectroscopy for biophysical investigations
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The torsional properties of DNA play an important role in cellular processes such as transcription, replication, and repair. To access these properties, a number of single-molecule techniques such as magnetic tweezers have been developed to apply torque to DNA and coil it. I will briefly refer to investigations of DNA-protein interactions using these techniques, and describe what has been learnt.
I will then focus on the development of novel magnetic techniques that go beyond standard magnetic tweezers, such as the magnetic torque tweezers[1] and the freely-orbiting magnetic tweezers[2]. These approaches allow one to quantify conjugate variables such as twist and torque. For example, the magnetic torque tweezers rely on high-resolution tracking of the position and rotation angle of magnetic particles in a low stiffness angular clamp. We demonstrate the experimental implementation of this technique and the resolution of the angular tracking. Subsequently, we employ this technique to measure the torsional stiffness *C* of both dsDNA molecules and RecA heteroduplex filaments.
Lastly, I will describe novel applications of the optical torque wrench[3,4]. The optical torque wrench is a laser trapping technique developed at Cornell capable of applying and directly measuring torque on microscopic birefringent particles via spin momentum transfer. We have focused on the angular dynamics of the trapped birefringent particle[4], demonstrating its excitability in the vicinity of a critical point. This links the optical torque wrench to non-linear dynamical systems such as neuronal and cardiovascular tissues, non-linear optics and chemical reactions, which all display an excitable binary ('all-or-none') response to input perturbations. Based on this dynamical feature, we devise a conceptually novel sensing technique capable of detecting single perturbation events with high signal-to-noise ratio and continuously adjustable sensitivity.

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P-687
Towards a single-molecule study of the AddAB helicase-nuclease with Optical Tweezers
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Double-stranded DNA breaks are a frequent form of nucleic acid damage that can lead to cell death, premature ageing or cancer. In bacteria, recombinational DNA break repair is initiated by a class of enzymes called helicase-nucleases. The prototypical member of this class in the model organism *Bacillus subtilis* is the protein complex AddAB. It processes a double-stranded end to a 3'-terminated single-stranded DNA overhang, thus creating a substrate for the subsequent repair steps [1, 2].
To study the role of force in the complex process of DNA repair initiation, we have set up and characterized a custom-built single-beam Optical Tweezers adapted from a published design [3]. Positions of trapped microspheres are measured by high-speed video microscopy as well as detection of backscattered laser light. Our novel instrumentation will enable us to investigate the real-time dynamics of repair processes catalyzed by the molecular motor AddAB. So far, proof-of-principle DNA force-extension measurements have been carried out. At present, we are endeavouring to determine the stalling force of AddAB, which should be larger than 4 pN, based upon observations from a complementary Magnetic Tweezers study (unpublished data).

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O-688
Protein dynamics and stability: universality *vs.* specificity
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Two seemingly conflicting properties of native proteins, such as enzymes and antibodies, are known to coexist. While proteins need to keep their specific native fold structure thermally stable, the native fold displays the ability to perform large amplitude motions that allow proper function. This conflict cannot be bridged by compact objects which are characterized by small amplitude vibrations and by a Debye density of low frequency modes. Recently, however, it became clear that proteins can be described as fractals;

namely, geometrical objects that possess self similarity. Adopting the fractal point of view to proteins makes it possible to describe within the same framework essential information regarding topology and dynamics using three parameters: the number of amino acids along the protein backbone N, the spectral dimension d_s and the fractal dimension d_f. The fractal character implies large amplitude vibrations of the protein that could have led to unfolding. We showed that by selecting a thermodynamic state that is "close" to the edge of stability against unfolding, nature has solved the thermostability conflict. Starting off from a thermal marginal stability criterion we reached a universal equation describing the relation between the spectral and fractal dimensions of a protein and the number of amino acids. Using structural data from the protein data bank (PDB) and the Gaussian network model (GNM), we computed d_f and d_s. for about 5,000 proteins (!) and demonstrated that the equation of state is well obeyed.

Proteins have been shown to exhibit anomalous dynamics. The anomalous behavior may, in principle, stem from various factors affecting the energy landscape under which a protein vibrates. We focused on the structure-dynamics interplay and showed how the fractal-like properties of proteins lead to such anomalous dynamics. We used diffusion, a method sensitive to the structural features of the protein fold and them alone, in order to probe protein structure. Conducting a large scale study of diffusion on over 500 PDB structures we found it to be anomalous, an indication of a fractal-like structure. Taking advantage of known and newly derived relations between vibrational dynamics and diffusion, we demonstrated the equivalence of our findings to the existence of structurally originated anomalies in the vibrational dynamics of proteins. More specifically, the time dependent vibrational mean square displacement (MSD) of an amino acid is predicted to be subdiffusive. The thermal variance in the instantaneous distance between amino acids is shown to grow as a power law of the equilibrium distance. The autocorrelation function in time of the instantaneous distance between amino acids is shown to decay anomalously. Our analysis offers a practical tool that may aid in the identification of amino acid pairs involved in large conformational changes.

More recently, we studied the effect of the hydrodynamic interaction between amino acids using a Zimm-type model. We computed the time-dependent MSD of an amino acid and the time-dependent autocorrelation function of the distance between two amino acids, and showed that these dynamic quantities evolve anomalously, similar to the Rouse-type behavior, yet with modified dynamic exponents.

We also studied the dynamic structure factor *S(k,t)* of proteins at large wavenumbers *k*, $kR_g >> 1$, with R_g the gyration radius, that are sensitive to the protein internal dynamics. We showed that the decay of *S(k,t)* is dominated by the spatially averaged MSD of an amino acid. As a result, *S(k,t)* effectively decays as a stretched exponential. We compared our theory with recent neutron spin-echo studies of myoglobin and hemoglobin for the Rouse and Zimm models of hydrodynamic friction.

In addition, I will mention two other projects currently underway: (i) A new elastic network model that accounts for the tensorial aspects of protein elasticity and is a combination of stretch-compress springs and bond-bending energies. (ii) The unfolding of a protein under the exertion of a large pulling force.

P-689

A discrete number of activity states constitute an efficient mechanism to regulate enzymatic activity

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Allosteric regulation of enzymatic activity is crucial for controlling a multitude of fundamental cellular processes. Yet the molecular level details underlying regulation often remain poorly understood. Here we employed single molecule activity studies to dissect the mechanistic origin of enzymatic activity regulation. As a model system we employed a lipase and measured its activity as a function of accessibility to surface tethered liposomes (1), which are known regulators of its activity. Our results surprisingly revealed that the lipase oscillates between 2 states of different activity. We accurately quantified for the first time both the interconversion rates between activity states and the inherent activity of these states. Based on these we calculated the energetic landscape of the entire reaction pathway and identified that regulatory interactions redistributed the probability to reside on preexisting enzymatic activity states but did not alter the activity of these states. Our findings provide the missing link between conformational and activity substates supporting and represent the first direct validation of the textbook hypothesis of conformational selection for regulation of enzymatic activity

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P-690

Proteomic identification of nucleoside diphosphate kinase as a novel cGMP-binding protein in *Arabidopsis*

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To identify the potential targets of cGMP in *Arabidopsis* plants we adopted a proteomic approach to isolate possible cGMP-binding proteins. Purification of soluble cGMP-binding proteins was performed using cGMP-agarose-based affinity chromatography procedure. Next eluted proteins were analyzed by SDS-PAGE which revealed ten bands. We focused the subsequent analysis on low-molecular peptides of 15, 16 and18 kDa which were bound cGMP more intensively. After 2D-IEF-PAGE of the proteins isolated by cGMP-agarose-affinity chromatography eight most abundant protein spots in the low-molecular area were visualized. These spots of interest were excised from the gel and in gel digested by trypsin. Then tryptic peptides were analyzed by MALDI-TOF mass spectrometry and identified as isoforms of nucleoside diphosphate kinase (NDPK) from *Arabidopsis*. Thus, our data suggest that NDPK is a potential target of cGMP signaling in *Arabidopsis*.

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P-691

Surface enhanced Raman spectroscopy for in vivo studies of erythrocytes

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For the first time we report a comparative approach based on surface enhanced Raman spectroscopy (SERS) and Raman spectroscopy to study different types of haemoglobin molecules in living erythrocytes. In erythrocytes there are two fractions of haemoglobin: cytosolic (Hb_c) and membrane-bound (Hb_m). The concentration of Hb_m is less then 0,5% and therefore it is impossible to study Hb_m with traditional optical techniques. Modifications of cellular membrane can affect conformation of Hb_m. Therefore, it can be used as a sensitive marker of pathologies.

Firstly, we investigated enhancement of SERS signal of Hb_m depending on Ag nanoparticles' size. We found that the intensity of SERS spectra of Hb_m and enhancement factor increase with the decrease in Ag nanoparticles' size. Secondly, we investigated the dependence of haemoporphyrin conformation in both Hb_c and Hb_m ion pH values. We observed different sensitivity of Hb_c and Hb_m to the pH and found that conformational movements of haemoporphyrin (vibrations of pyrrol rings and side radicals) in Hb_m are sterically hampered comparably with Hb_c.

Our observation is an evidence of a benefit of application of surface enhanced Raman spectroscopy to investigate properties of the Hb_m in erythrocytes and provide new information about conformational changes and functional properties of Hb_m.

P-692

Single-molecule FRET study of RNA free energy landscape: cooperative effects of nucleotide modifications and cations

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RNA nanotechnology is an emerging field with high potential for nanomedicine applications. However, the prediction of RNA three-dimensional nanostructure assembly is still a challenging task that requires a thorough understanding the rules that govern molecular folding on a rough energy landscape. In this work, we present a comprehensive analysis of the free energy landscape of the human mitochondrial $tRNA^{Lys}$, which possesses two different folded states in addition to the unfolded one. We have quantitatively analyzed the degree of RNA tertiary structure stabilization, firstly, for different types of cations,[1] and, secondly, for several naturally-occurring nucleotide modifications in the structural core of the $tRNA^{Lys}$.[2,3] Thus, notable variations in the RNA binding specificity was observed for the divalent ions of Mg^{2+}, Ca^{2+} and Mn^{2+}, that can be attributed to their sizes and coordination properties to specific ligands. Furthermore, we observed that the presence of m^2G10 modification together with the principal stabilizing m^1A9 modification facilitates the RNA folding into the biologically functional cloverleaf shape to a larger extent than the sum of individual contributions of these modifications.

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P-693

Brownian motions of peptide/MHC complex define the activation of T cells

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TCR should recognize conformations of peptide/MHC with rigid form. Recently, however, Unanue et al. reported that certain T cells (Type B) only recognize MHC with loosely bound peptide. In order to elucidate the mechanism of the recognition, we used diffracted X-ray tracking method (DXT) that monitors real-time movements of individual proteins in solution at the single-molecule level. We found that peptides move distinctly from $I\text{-}A^k$, and the rotational motions of peptides correlate with the type B T cell activation. In the case of diabetogenic $I\text{-}A^{g7}$, immediately after peptide exchange, all the peptides moves magnificently but the motion ceased in a week, then new ordered motion appears; the rotational motion of peptides correlate to T cell activation, which is analogous to the peptide in $I\text{-}A^k$. The rotational motion of peptides may create transient conformation of peptide/MHC that recognized by a population of T cells. DXT measurement of peptide/MHC complex well correlated to other biological phenomenon too. Our finding is the first observation that fluctuations at the level of Brownian motion affect to the functions of proteins.

P-694

Quaternary structure of SecA in solution and bound to SecYEG probed at the single molecule level

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Dual-color fluorescence-burst analysis (DCFBA) was applied to measure the quaternary structure and high affinity binding of the bacterial motor protein SecA to the protein-conducting channel SecYEG reconstituted into lipid vesicles. DCFBA is an equilibrium technique that enables the direct observation and quantification of protein-protein interactions at the single molecule level. SecA binds to SecYEG as a dimer with a nucleotide- and preprotein-dependent dissociation constant. One of the SecA protomers binds SecYEG in a salt-resistant manner, while binding of the second protomer is salt-sensitive. Since protein translocation is salt-sensitive we conclude that the dimeric state of SecA is required for protein translocation. A structural model for the dimeric assembly of SecA while bound to SecYEG is proposed based on the crystal structures of the *Thermatoga maritima* SecA-SecYEG and the *Escherichia coli* SecA dimer.

Highlights

- DCFBA is a flurorescence based single molecule technique that allows assessment of the stoichiometry of ligands bound to membrane receptors
- Dimeric SecA binds asymmetrically to the protein-conducting membrane channel SecYEG
- Monomeric SecA binds SecYEG but dimeric SecA is required for protein translocation
- Protein translocation depends on receptor cycling of the dimeric SecA

P-695

Helix specific electrostatic effects in DNA braiding and supercoiling

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If the DNA charge is sufficiently neutralized by counter-ions, electrostatic interactions between helical charge patterns can cause attraction [1]. Helix specific interactions also cause tilt, in one direction, between two DNA fragments [1]. In braids and supercoils, this impetus to tilt breaks positive-negative supercoil symmetry. We show that these effects may cause spontaneous braiding of two molecules, lowering the DNA pairing energy [2]. The pairing is more energetically favourable for homologues (same base pair text) than for nonhomologous pairs. This might explain pairing between only homologues observed in NaCl solution [3]. Also, we construct a simple model for a closed loop supercoil, including chiral electrostatic interactions. There are very interesting effects, for sufficient charge neutralization and groove localization of counter-ions. i.) Positive super-coils are more energetically favourable than negative ones. ii.) A transition between loosely and tightly wound supercoils as one moves from negative to positive values of the supercoiling density. iii.) In positive super-coils the chiral interaction underwinds DNA.

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P-696

Binding and conformational changes of VWF under shear

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Von Willebrand Factor (VWF) is a large multimeric protein that is crucial for the force sensing cascade triggering primary hemostasis. It mediates binding of activated thrombocytes to injured epithelial tissue and serves as a transporter for coagulation factor VIII. While it was shown that the hemostatic activity of VWF is affected by shear stress [1], the exact impact that shear forces have on the inflammatory cascade remains unclear. It is assumed that hydrodynamic forces lead to partial unfolding of VWF, which in consequence exposes more binding sites.

In order to observe shear-induced changes of the protein's functionality, we measure conformational changes of VWF under flow with fluorescence correlation spectroscopy (FCS). We aim to measure the degree of uncoiling of VWF multimers under various buffer conditions, e.g. in the presence of colloids, vesicles or platelets. As only large multimers show significant hemostatic activity we intend to monitor the molecular weight distribution of VWF. Shifts in this distribution indicate various pathological conditions making our multimer analysis a fast diagnostical tool for VWF-related diseases. This will serve as a basis for studies of VWF binding to collagen, FVIII, GPIb, vesicles and membrane-coated nanoparticles under shear flow.

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P-697
Nanomechanics of neural junction—stretching FNIII domains of human contactins
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Data on mechanical properties of medically important proteins located in neural junctions are very limited. Contactins (CNTN) and paranodin proteins, located in extracellular part of Ranvier nodes, are important for proper brain wiring. Here we study a new series of FNIII modules from human CNTN-1 and -4 using a single molecule AFM force spectroscopy and advanced, all-atom steered molecular dynamics (SMD) computer simulations. Mutations in CNTNs are responsible for numerous brain disorders including autism or pathological development of odor maps. Perhaps mechanical properties of individual FNIII mutated protein modules are compromised, thus we address this problem. A comparison of our AFM force spectra with those of reference proteins will be presented [1-2], and the molecular level interpretation FNIII nanomechanics, based on our SMD data will be given. We believe that these data should help to understand a role of CNTN in regulation of sodium ion channels in both normal and autistic subjects.
Supported in part by Polish Ministry of Education and Science, grant no. N202 262038, the Computational Center TASK in Gdansk and license for Accelrys software.

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P-699
Building and visualizing DNA-motors using single-molecule fluorescence spectroscopy
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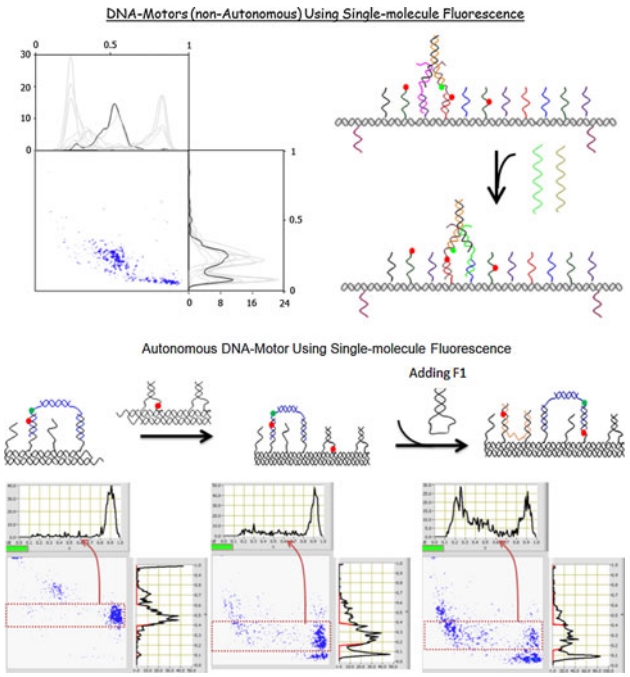
Recent achievements in rational DNA-motors engineering demonstrate the possibility to design nano-motors and nano-robots capable of performing externally controlled or programmed tasks. A major obstacle in developing such a complex molecular machine is the difficulty in characterizing the intermediates, the final products and their activity. Typically, non *in-situ* Gel and AFM and *in-situ* bulk fluorescence methods are used. I will present two DNA-motors recently developed and studied using *in-situ* single-molecule fluorescence resonance energy transfer (smFRET), alternating laser excitation (ALEX) and total internal reflection fluorescence spectroscopy (TIRF), and will demonstrate that these methods can improve the way we design, construct, measure and understand highly complex DNA-based machines.
A motor made of bipedal DNA-walker, which walks on a DNA track embedded on a DNA-origami, capable of long walking distance and maintaining structural stability, will be presented. The motor is non-autonomous; it receives ss-DNA fuel/anti-fuel commands from outside (as in Shin & Pierce, JACS, 2004). The motors assembly stages and single-motor's walking steps are monitored using smFRET.

The second motor is based on published bipedal autonomous DNA-motor (Seeman, Science 2009). It is characterized by coordinated activity between the different motor domains leading to processive, linear and synchronized movement along a directionally polar track. To prove that the motor indeed walks, the authors chemically froze the motor at each step and use a complicated radioactive Gel assay. I will demonstrate that using single-molecule approach, we are able to directly and *in-situ* measure single-motor's movements in few simple experimental steps, and measure its structural dynamics and kinetics.



P-700
Translation by a single eukaryotic ribosome
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Using single molecule total internal reflection fluorescence microscopy, we observed translation of a short messenger RNA (mRNA) strand by single eukaryotic ribosomes. The ribosome-mRNAs complexes are fixed to a microscope coverslip through the mRNA, and mRNAs are located through fluorescently labelled oligonucleotides hybridized to it downstream start codon. Because of the ribosome helicase activity, the double strand formed by the oligonucleotide and the mRNA is opened while the ribosome translates this region of the mRNA. Thus, the loss of the fluorescence signal allows us to measure the distribution of translation speed of single ribosomes. Careful attention was given to photobleaching for the data analysis. This experiment opens the door to the study of eukaryotic translation at the single molecule level.

P-701

Single molecule cut and paste for protein based functional assembly

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Single-molecule cut-and-paste surface assembly (SMCP) has formerly been employed in the controlled deposition of individual fluorophores in well-defined nanometer sized patterns [1]. The technique allows for the creation of patterns of arbitrary shape and with arbitrary numbers of single molecules consisting of multiple species. The accuracy has been shown to be ± 10 nm with the given spacer and DNA sequence lengths [2]. SMCP has been also used to build up a Biotin scaffold that Streptavidin-coated nanoparticles could bind to [3].
Utilizing specific molecular interactions, for example between DNA-binding proteins and DNA or antibodies and antigens, this technique is capable of providing a scaffold for the controlled self-assembly of functional complexes. Furthermore, this allows for the introduction of SMCP into protein science.
We aim to employ DNA-binding Zinc-finger variants and GFP-binding nanobodies as shuttle-tags fused to the proteins of interest. Thus a fully expressible system that can be used for the step-wise assembly of individual building blocks to form, for example, large enzyme complexes or protein networks, is provided.

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P-703

Force spectroscopy characterization of fibrinogen-erythrocyte binding and its conditioning by aging

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Erythrocyte hyperaggregation, a cardiovascular risk factor, has been associated to high plasma concentrations of fibrinogen. Using atomic force microscopy (AFM)-based force spectroscopy measurements, we have recently identified the erythrocyte membrane receptor for fibrinogen, an integrin with a $\alpha 3$ or $\alpha 3$-like subunit [1]. After this, we extended the study to the influence of erythrocyte aging on fibrinogen binding [2]. Force spectroscopy measurements showed that upon erythrocyte aging, there is a decrease of the binding to fibrinogen by decreasing the frequency of its occurrence (from 18.6% to 4.6%) but not its force. This observation is reinforced by zeta-potential and fluorescence spectroscopy measurements. Knowing that younger erythrocytes bind more to fibrinogen, we could presume that this population is the main contributor to the cardiovascular diseases associated with increased fibrinogen blood content, which disturbs the blood flow. Our data also show that sialic acid residues on the erythrocyte membrane contribute for the interaction with fibrinogen, possibly by facilitating the binding to its receptor.

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P-704

Structure-function investigation of a novel dendrimeric and lipidated antimicrobial peptide

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Antimicrobial peptides are usually polycationic and amphiphilic with high affinity for bacterial membranes. In order to characterize their therapeutic potential it is crucial to disclose which properties of the peptide/lipids are important for target selectivity, and to examine the peptide structure and its association with lipid bilayers. In this work, first experiments have been carried out on a promising peptide called SB056, which might represent the basis for developing a novel class of antibiotics. With the goal of enhancing the activity of a new semi-synthetic sequence, two identical peptides (WKKIRVRLSA) were assembled via a lysine-linker, carrying also an octanoyl-lipid anchor. A highly active compound was obtained, but its structure and mode-of-action remain unexplored. This dendrimeric peptide and its linear deca-peptide counterpart are being studied in parallel to highlight the relevant properties and differences between dendrimeric structure and the sequence. Monolayer intercalation is investigated with microtensiometry, Fluorescence spectroscopy is applied to study thermodynamics and kinetics of the binding process. Circular dichroism, NMR and MD simulations are employed with the aim of elucidating the 3D structure in the membrane-bound state.

P-705

Coronavirus nsp7-nsp8 complex formation

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The capability of proteins to build structures via self-organization is fascinating biophysicists since decades. With the

advent of single-molecule methods, namely fluorescence correlation spectroscopy (FCS) and fluorescence resonance energy transfer (FRET), the process of complex formation is becoming accessible to direct observation.
Coronaviruses (CoV) are enveloped positive-stranded RNA viruses. For SARS-CoV, it was shown that coronaviruses encode a RNA-dependent RNA-polymerase (RdRp) build from non-structural protein 7 (nsp7) and non-structural protein 8 (nsp8). This hexadecameric nsp7-nsp8 complex is a hollow, cylinder-like structure assembled from eight copies of nsp8 and held together by eight nsp7 molecules [1,2].
We are aiming at understanding the assembly process and conformational changes of the complex for the related Feline Coronavirus. First results implicate that nsp8 alone forms a dimer, where interchain FRET is more efficient than intrachain FRET. For the complex the results indicate that nsp7-nsp8 form a heterodimer which is different from SARS-CoV.
Our experiments highlight the potential of single-molecule FRET for the study of protein complex formation.

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P-706
Torsional motion analysis of group II chaperonin using diffracted X-ray tracking
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Diffracted X-ray Tracking (DXT) has been considered as a powerful technique for detecting subtle dynamic motion of the target protein at single molecular level. In DXT, the dynamics of a single protein can be monitored through trajectory of the laue spot from the nanocrystal which was labeled on the objective protein.
In this study, DXT was applied to the group II chaperonin, a protein machinery that captures an unfolded protein and refolds it to the correct conformation in an ATP dependent manner. A mutant group II chaperonin from Thermococcus strain KS-1 with a Cys residue at the tip of the helical protrusion was immobilized on the gold substrate surface and was labeled with a gold nanocrystal. We monitored diffracted spots from the nanocrystal as dynamic motion of the chaperonin, and found that the torsional motion of the chaperonin in the presence of ATP condition was 10 times larger than that in the absence of ATP condition. And UV-light triggered DXT study using caged ATP revealed that the chaperonin twisted counterclockwisely (from the top view of chaperonin) when the chaperonin closed its chamber, and the angular velocity from open to closed state was 10 % faster than that from closed to open state.

P-707
Single molecule fluorescence measurements of soluble tau oligomers
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Peptides or proteins may convert (under some conditions) from their soluble forms into highly ordered fibrillar aggregates. In vivo such transitions can lead to neurodegenerative disorders such as Alzheimer's Disease. Alzheimer's Disease is characterised by the extracellular deposition of Abeta peptide in amyloid plaques, and the intracellular formation of neurofibrillary tangle (NFT) deposits within neurons, the latter correlating well with disease severity. The major constituent of NFT deposits are paired helical filaments (PHF) composed of a microtubule-associated protein known as tau. Studying the process by which tau forms these large aggregates may be an essential step in understanding the molecular basis of Alzheimer's Disease and other Tauopathies. We have applied a two-colour single molecule fluorescence technique, and single molecule intermolecular FRET measurements to study the soluble oligomers of tau which are formed during the aggregation and disaggregation of PHF's.

P-708
Histone fold modifications control nucleosome unwrapping and disassembly
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The nucleosome structure compacts the genome by wrapping 147 base pairs of DNA 1.65 times around a histone protein octamer, while alterations of this structure such as partial DNA unwrapping and nucleosome disassembly regulate gene expression, replication, and repair. We examined the regulation of these structural changes by histone post-translational modifications (PTMs) in key regions of histone-DNA interface. Precise histone PTMs were introduced into nucleosomes by preparing semi and fully synthetic histones by expressed protein ligation and native chemical ligation. Single molecule mechanical measurements of nucleosome arrays determined that only PTMs within the nucleosome dyad region increase the nucleosome disassembly rate. In contrast, Förster resonance energy transfer (FRET) analysis and enzyme kinetic experiments with mononucleosomes show 2-to-3 fold increase in DNA unwrapping only for PTMs located within the entry/exit region. Our studies suggest that the nucleosome regions that control DNA unwrapping and nucleosome disassembly are decoupled, where DNA histone contacts near the dyad regulate disassembly and the DNA entry-exit region regulates DNA unwrapping.

P-709

Nanomechanical manipulation of Mason-Pfizer monkey retroviral RNA fragment with optical tweezers

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Mason-Pfizer monkey virus (MPMV) is an excellent model for the analysis of retrovirus assembly and maturation. However, neither the structure of the viral RNA, nor its modulation by capsid-protein binding are exactly known. To explore the structure of the MPMV genome, here we manipulated individual molecules of its packaging signal sequence with optical tweezers.

The 207-base-long segment of MPMV RNA corresponding to the packaging signal, extended on each side with 1200-base-long indifferent gene segments for use as molecular handles, was cloned into a pET22b vector. RNA was synthesized in an in vitro transcription system. RNA/DNA handles were obtained by hybridization in a PCR with complementary DNA initiated with primers labeled with either digoxigenin or biotin. The complex was manipulated in repetitive stretch and relaxation cycles across a force range of 0-70 pN. During stretch, transition occurred which increased the RNA chain length and likely corresponds to unfolding. The length gain associated with the unfolding steps distributed across three main peaks at $\sim$13, 20, 32 nm, corresponding to $\sim$35, 57, 85 bases, respectively. Often reverse transitions were observed during mechanical relaxation, indicating that refolding against force proceeds in a quasi-equilibrium process.

P-710

Structural investigation of GPCR transmembrane signaling by use of nanobodies

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In 1993, scientists at the Vrije Universiteit Brussel discovered the occurence of *bona fide* antibodies devoid of light chains in *Camelidae*. The small and rigid recombinant antigen binding fragments (15kD) of these heavy chain only antibodies – known as VHHs or Nanobodies – proved to be unique research tools in structural biology.

By rigidifying flexible regions and obscuring aggregative surfaces, nanobody complexes warrant conformationally uniform samples that are key to protein structure determination by X-ray crystallography:

- Nanobodies bind cryptic epitopes and lock proteins in unique native conformations
- Nbs increase the stability of soluble proteins and solubilized membrane proteins
- Nbs reduce the conformational complexity of soluble proteins and solubilized membrane proteins
- Nbs increase the polar surface enabling the growth of diffracting crystals
- Nbs allow to affinity-trap active protein

I will focus my talk on the use of Nbs for the structural investigation of GPCR transmembrane signaling to illustrate the power of the Nanobody platform for generating diffracting quality crystals of the most challenging targets including GPCRs and their complexes with downstream signaling partners.

P-713

Dynamics of the Type I interferon receptor assembly in the plasma membrane

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Type I Interferons (IFN) are key cytokines in the innate immune response and play a critical role in host defense. All IFNs bind to a shared cell surface receptor comprised of two subunits, IFNAR1 and IFNAR2. Detailed structure-function analysis of IFNs has established that the IFN-receptor interaction dynamics plays a critical role for signalling specificities. Here we have explored the dynamics of receptor diffusion and IFN assembly in living cells. By using highly specific orthogonal posttranslational labelling approaches combined with TIRF-microscopy we probed the spatio-temporal dynamics of receptor diffusion and interaction in the plasma membrane of live cells on the single molecule level. For this purpose, we employed posttranslational labelling with photostable organic fluorophores. This allowed us to map diffusion and lateral distribution of IFNAR1 and IFNAR2 with very high spatial and temporal resolution by using single particle tracking (SPT) and single molecule localization imaging. Observed events of "transient confinement" and co-localization with the membrane-proximal actin-meshwork suggest partitioning of IFNAR1/2 in specialized microcompartments. This will be investigated in terms influence on receptor assembly and recruitment of cytoplasmic effector proteins.

O-714

Cytoplasmic dynein moves through uncoordinated action of the AAA+ ring domains

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Cytoplasmic dynein is a homodimeric AAA+ motor that moves processively toward the microtubule minus end. The mechanism by which the two catalytic head domains interact and move relative to each other remains unresolved. By tracking the positions of both heads at nanometer resolution, we found that the heads remain widely separated and move independently along the microtubule, a mechanism different from that of kinesin and myosin. The direction and size of steps vary as a function of interhead separation. Dynein processivity is maintained with only one active head, which drags its inactive partner head forward. These results challenge established views of motor processivity and show that dynein is a unique motor that moves without strictly coordinating the mechanochemical cycles of its two heads.

O-715
Self-controlled monofunctionalization of quantum dots and their applicaitons in studying protein-protein interaction in live cells
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Individual proteins labeled with semiconductor nanocrystals (quantum dots, QD) greatly facilitate studying protein-protein interactions with ultrahigh spatial and temporal resolution. Multiplex single molecule tracking and imaging require monovalent quantum dots (mvQD) capable of orthogonally labeling proteins with high yield. For this purpose, we prepared monovalance QD-trisNTA by a chemical conjugation method. Our results indicated that monovalent QD-trisNTA was obtained in high yield by restricting the coupling by means of electrostatic repulsion. Monovalent functionalization of the QD-trisNTA was confirmed by assays *in vitro* and *in vivo*. Two-color QD tracking of interferon receptors IFNAR1 and IFNAR2 based on mvQD-trisNTA were realized on live cell [1].To broaden the multiplex toolbox of mvQDs, we extended the electrostatic-repulsion induced self-control concept for mono-functionalizing quantum dots with different affinity moieties. As a first instance, we used negatively-charged biotin peptide to produce QD with biotin mono-functionalization. We confirmed our approach was a general method to rend QD monovalent by single molecule assays based on stepwise photobleaching. These mvQDs facilitate obtaining spatiotemporal information of IFNARs' organization in live cells. By orthogonal labeling U5A cells stably expressing IFNAR2 at low level with biotin mvQD and mvQD-trisNTA-IFN, we verified colocalization and colocomotion of individual IFN and IFNAR2 at minute scale. Combined with super-resolution imaging of IFNARs' cytosolic effecter STAT2, we observed the dynamic coming-and-going contact between the microcompartments of IFNAR2 and STAT2.

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P-716
Establishing the composition of alpha-synuclein oligomers using single-molecule photobleaching
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The neuronal protein alpha-synuclein is considered to play a critical role in the onset and progression of Parkinson's disease. Fibrillar aggregates of alpha-synuclein are the main constituents of the Lewy bodies that are found in the brains of Parkinson patients. However, there is growing evidence suggesting that oligomeric aggregates are significantly more toxic to cells than fibrillar aggregates. Very little is known about the structure and composition of these oligomeric aggregates.
We present results using single-molecule photobleaching approaches to determine the number of monomeric subunits constituting the oligomers. Our results show that the oligomers have a narrow size distribution, consisting of $\sim$13-20 monomers per oligomer. Fluorescence correlation spectroscopy data confirm the narrow size distribution and additionally indicate a very loose packing of the oligomers. In combination with bulk fluorescence spectroscopy results of tryptophan containing mutants of alpha-synuclein, we present a structural model for the alpha-synuclein oligomer.

Micro and nanotechnology

P-717
Directly assessment of drug release dynamics from gold nanoparticles
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Gold colloids are widely used for *in vitro* and *in vivo* imaging. Compared to the traditional optical tags SERS-coded nanoparticles show a narrow emission bandwidth with structured spectra typical of the molecule used, a wider excitation bandwidth, higher emission intensity, a better photo-stability, and a lower toxicity.
This is why in cancer therapy, besides being considered good tools for the delivery of anti-tumor drugs, AuNP can be also good optical tags for the analyses of both NP localization by laser scanning microscopy and the process of drug release inside the cells by Raman.
In our work we used 10 nm diameter AuNP loaded with Rhodamine 6G, a molecule with a high Raman and fluorescence efficiency, and with a chemical structure similar to Doxorubicin, the antitumoral drug used in our system. The data showed that AuNP are internalized by cells and SERS can be performed.
10 nm and 60 nm diameter AuNP loaded with Doxorubicin were incubated at different time points with A549 cell line (human adenocarcinomic alveolar basal epithelial cells). Only 60 nm AuNP showed intense Raman emission typical of the doxorubicin phonon transitions.
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P-720

Effect of non-modified and Fenton-modified nanodiamond powder on human endothelial cells

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In recent years biomedical applications of diamond nanoparticles have become of significant interest, which rises questions of their biocompatibility and mechanisms of interactions with cells. The aim of this study was to compare the effect of non-modified diamond nanoparticles (DNPs) and DNPs modified by the Fenton reaction on human endothelial cells.

DNPs (<10 nm particle size, SIGMA) were modified by the Fenton reaction introducing surface –OH groups. Immortalized human endothelial cells (HUVEC-ST) were incubated with 2-100 μg/ml DNPs in the OPTIMEM medium.

Diamond nanoparticles modified by the Fenton reaction had smaller hydrodynamic diameter estimated by dynamic light scattering and the surface potential (zeta potential) measured using laser-Doppler electrophoresis. They were more cytotoxic as evaluated by the MTT reduction assay.

DNPs augmented generation of reactive oxygen species in the cells, estimated by oxidation of 2',7'-dichlorofluorescin, the effect being higher for the Fenton-modified DNPs after 48-h incubation. Cellular production of nitric oxide, estimated with DAF-FM, was also affected by DNPs; after 72h, Fenton-modified OH, in contrast to non-modified diamond, decreased NO production.

Diamond nanoparticles affected also the cellular level of glutathione and activities of main antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase).

P-721

Time-resolved FTIR difference spectroscopy of bacteriorhodopsin under vibrational control

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We aim at investigating how photoreactions of proteins can be controlled by means of intense THz radiation tuned in resonance to specific vibrational modes, much in analogy to coherent control experiments conducted by fs NIR laser pulses [1]. For this we will combine a time-resolved IR difference spectroscopic setup with uniquely intense, tunable narrow bandwidth THz radiation (3 – 280 μm) at the ps beamline of the THz free electron laser FELBE. These experiments will be performed on bacteriorhodopsin (bR) which is the sole protein of the purple membrane of the archaebacterium *Halobacterium salinarum* [2]. Upon illumination, the chromophore retinal isomerizes around the C_{13}-C_{14} double bond [3] and bR pumps a proton from the cytoplasmic to the extracellular side. This proton gradient is used by the bacterium to drive photosynthetic ATP production under low oxygen tension [4]. In our experiment, the photoreaction is initiated by a visible laser pulse as in standard experiments, but then the sample will be irradiated by a THz pulse from the free electron laser tuned into resonance with low-energy vibrational modes which is supposed to influence the photoreaction [1]. Such vibrational control will be monitored by time-resolved FTIR spectroscopy using the step-scan technique [5].

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P-722

Structural stability of surface-adsorbed liposomes

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Liposomes are increasingly studied as nanoscale drug delivery systems and biomembrane models. However the exact structure dynamics and mechanical behavior of liposomes is little known. Atomic force microscopy (AFM) is a powerful tool to characterize nanoscale morphology and enables the mechanical manipulation of submicron-sized vesicles. A drawback of AFM, however, is that liposomes may flatten and rupture on substrates to form patches or supported planar bilayers (SPB).

Our aim was to obtain better understanding of factors affecting liposomes on substrates and find experimental conditions at which liposomes preserve their structural integrity.

In the presence of divalent cations DPPC liposomes formed SPB on mica. Vesicles sedimented subsequently preserved their integrity and showed stronger attachment to SPB. In addition to cross-bridging lipid head groups, divalent cations influence the surface charge of liposomes, thereby modulating liposome-substrate and liposome-liposome interfacial interactions. Preserved vesicles stabilized by divalent cations may provide a unique experimental system for studying membrane-protein interactions.

P-724
Micro-viscosimeter generated and manipulated by light
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A micron sized viscometer was fabricated using the Couette type geometry that is capable of measuring the complex viscosity of fluids. The viscometer was produced by two photon polymerization of SU8 photopolymer using a femtosecond laser system, a high NA objective and a piezo translator stage. The viscometer was manipulated by holographic optical tweezers and operated in the 0.005-1 Hz frequency range. Video analysis algorithm was used to evaluate our measurements. We tested the viscometer with water-glycerol solutions.

P-725
Shaping substrates at the nanoscale for single cell characterization
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One of the main reasons for lack of reliability in protein analysis for disease diagnostics or monitoring is a lack of test sensitivity. This is because, for many tests, to be reliable, they need to be performed on a homogeneous, and therefore very small, sample. Current in-vitro techniques fail in accurately identifying small differences in protein content, function and interactions starting from samples constituted of few or even single cells. A nanotechnology approach may overcome the current limits in low abundance protein detection.
We aim at designing a microwell device for the trapping (in native environment) and the parallel characterization of rare cells (e.g. adult stem cells). Such versatile device, based on soft and nanolithography, will promote cell adhesion and viability on differently functionalized bio-compatible materials, allowing for the morphological characterization of the cells, at a single cell level. In parallel, by facing our microwell device with a protein nanoarray, produced via atomic force microscopy nanolithography, we can run proteomic studies at a single/few cells level.
Moreover, we could foresee the possibility to deliver different stimuli to each cell, correlating the changes in chemistry/morphology with the protein profile at a single cell level.

P-726
Mechanical characterisation of hollow silicon microprobes fabricated by Deep Reactive Ion Etching
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Summary: Hollow in-plane microprobes were fabricated in silicon by deep reactive ion etching. Sealed microchannels in the probeshaft were formed by a modified Buried Channel Technology (BCT). Besides the improvement in the fabrication process, the influence of channel design on the mechanical stability of the probes was also characterized by the Stress-Strain Module of COMSOL Multiphysics simulation code. An in-vivo test was carried out in order to reveal the robustness of the device.

O-727
SSM-based electrophysiology: transport mechanism and pH-regulation of the Na^+/H^+ antiporter NhaA from E. coli
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Using an electrophysiological assay the activity of NhaA was tested in a wide pH range from pH 5.0 to 9.5. Forward and reverse transport directions were investigated at zero membrane potential using preparations with inside out and right side out oriented transporters with Na^+ or H^+ gradients as the driving force. Under symmetrical pH conditions with a Na^+ gradient for activation, both the wt and the pH-shifted G338S variant exhibit highly symmetrical transport activity with bell shaped pH dependencies, but the optimal pH was shifted 1.8 pH units to the acidic range in the variant. In both strains the pH dependence was associated with a systematic increase of the K_m for Na^+ at acidic pH. Under symmetrical Na^+ concentration with a pH gradient for NhaA activation an unexpected novel characteristic of the antiporter was revealed; rather than being down regulated it remained active even at pH as low as 5.
These data allowed to advance a transport mechanism based on competing Na^+ and H^+ binding to a common transport site and to develop a kinetic model quantitatively explaining the experimental results. In support of these results both alkaline pH and Na^+ induce the conformational change of NhaA associated with NhaA cation translocation as demonstrated here by trypsin digestion. Furthermore, Na^+ translocation was found to be associated with the displacement of a negative charge. In conclusion, the electrophysiological assay allowed to reveal the mechanism of NhaA antiport and sheds new light on the concept of NhaA pH regulation.

O-728
Swimming motility of bacteria near solid surfaces
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Swimming motility is widespread among bacteria. However, in confined or structured habitats bacteria often come in contact with solid surfaces which has an effect on the swimming characteristics.
We used microfabrication technology to quantitatively study the interaction of swimming cells with solid boundaries. We tracked bacteria near surfaces with various engineered topologies, including flat and curved shapes. We were able to study several surface related phenomena such as hydrodynamic trapping and correlated motion.
We think that our results may help to understand how physical effects play a role in surface related biological processes involving bacteria such as biofilm formation.

P-729
Cell labeling efficiency of oppositely charged magnetic iron oxide nanoparticles—a comparative study
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The interaction of nanomaterials with cells is a key factor when considering their translocation into clinical applications. Especially an effective accumulation of nanoparticles inside certain tissues is beneficial for a great number of applications. Predominantly size, shape and surface charge of nanoparticles influence their cellular internalization and distribution. To investigate this, two series of maghemite (γ-Fe_2O_3) nanoparticles were synthesized either *via* aqueous coprecipitation or *via* thermal decomposition of organometallic precursor molecules. Size and the spherical shape of both nanoparticle types were kept constant whereas the charge was changed by modifying the surface of the nanoparticles with polymers of opposite charge, in detail poly(ethylene imine) (PEI) and a polymaleic anhydride derivative (PMA). The positively and negatively charged γ-Fe_2O_3 nanoparticles were characterized with respect to size, zeta potential, colloidal stability and magnetic properties. Furthermore, the uptake rate and localization of both formulations into A549 carcinoma cells after fluorescent labeling of the carriers as well as the resulting alteration in MR-relaxation times were evaluated.

O-730
Surface-Enhanced InfraRed Absorption Spectroscopy (SEIRAS) of membrane protein monolayers
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Membrane proteins are the target of more than 50% of all drugs and are encoded by about 30% of the human genome. Electrophysiological techniques, like patch-clamp, unravelled many functional aspects of membrane proteins but usually suffer from poor structural sensitivity. We have developed Surface Enhanced Infrared Difference Absorption Spectroscopy (SEIDAS)[1,2] to probe potential-induced structural changes of a protein on the level of a monolayer. A novel concept is introduced to incorporate membrane proteins into solid supported lipid bilayers in an orientated manner via the affinity of the His-tag to the Ni-NTA terminated gold surface[3]. Full functionality of surface-tethered cytochrome c oxidase is demonstrated by cyclic voltammetry after binding of the natural electron donor cytochrome c. General applicability of the methodological approach is shown by tethering photosystem II to the gold surface[4]. In conjunction with hydrogenase, the basis is set towards a biomimetic system for H_2-production. Recently, we succeeded to record IR difference spectra of a monolayer of sensory rhodopsin II under voltage-clamp conditions[5]. This approach opens an avenue towards mechanistic studies of voltage-gated ion channels with unprecedented structural and temporal sensitivity. Initial vibrational studies on the novel light-gated channelrhodopsin-2 will be presented[6].

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P-731
Probing biomass-chromatographic bead interactions by AFM force spectroscopy
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In expanded bed adsorption (EBA), bioproducts are purified from an unclarified fermentation broth by their adsorption on chromatographic beads in a fluidized bed. The unspecific deposition of biomass onto the adsorbent matrix can severely affect the process performance, leading to a poor system hydrodynamics which then decreases the success of this unit operation.
To quantify the bead-biomass interactions different chromatographic beads are attached to AFM cantilevers, and force spectroscopy experiments are performed with these colloidal probes on model surfaces and cells in solution. The experiments are conducted under varying conditions to study

the influence of e.g. pH and ionic strength on various chromatographic bead - biomass combinations. To analyze the force curves, possible elastic contributions, e.g. from deforming cellular membranes, have to be decoupled from the interaction forces. Then, bead-biomass interactions will be modeled using (extended) DLVO theory and resulting data can also be compared to real-life EBA processes. The project aims for a better understanding of the interaction forces in chromatography and might help to improve the process quality of EBA.

O-732
Multifunctional magnetic nanoparticles for cell imaging

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Long-term non-invasive *in vivo* monitoring of the survival, migration, homing and fate of transplanted cells is of key importance for the success of cell therapy and regenerative medicine. Tools for *in vivo* magnetic resonance (MR) imaging of labeled cells are therefore being developed. We have prepared superparamagnetic iron oxide nanoparticles by the coprecipitation of Fe(II) and Fe(III) salts and oxidation. To stabilize the particles and to facilitate their internalization by the cells, the nanoparticles were coated with several novel low- and high-molecular weight compounds including D-mannose, poly(L-lysin), poly(*N,N*-dimethylacrylamide) and dopamine-hyaluronate conjugate. The surface-modified magnetic nanoparticles were thoroughly characterized by a range of physico-chemical methods, which proved the presence of the coating on the particles. The particles were then investigated in stem cell experiments in terms of real time cell proliferation analysis, viability, labeling efficiency and differentiation. The iron oxide concentration of the labeled cells was assessed using MR relaxometry. The advantages/disadvantages of particular iron oxide coatings will be discussed and the optimal coating suggested. Excellent contrast was achieved by labeling the cells with dopamine-hyaluronate-coated nanoparticles.
Support of the AS CR (No. KAN401220801) is acknowledged.

P-733
Selective bio-functionalization of electrodes inside micro and nano fluidic channels

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In the last decades the interest towards the fabrication of innovative bio-sensors with improved sensitivity and reliability for medical-diagnostics applications has been constantly risen. Among the different techniques, microfluidic systems are playing a major role. In order to detect extremely low concentrations of biomolecules (pM and fM), attention should be placed on the controlled, selective functionalization of micro- and nano-channels.
In this work we propose a new approach to functionalize gold patches inside fluidic channels. We start from self-assembled monolayers (SAMs) of thiolated molecules on a gold electrode deposited inside the channel. Then, by using an electrochemical approach [1,2] we remove molecules from the SAM at selected locations, by applying a negative voltage to the electrode. The newly exposed gold surface can be re-functionalized by using a thiolated biomolecule (i.e an antibody) capable to bind specific proteins flowing inside the channel. The cycle can be applied to other electrodes in the microfluidic system, creating a multiplexing device which, as we will show, can differentially measure ionic current flows in different channels.

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P-734
Microtools made by two-photon polymerization for optical tweezers systems used in biological manipulation experiments

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Optically actuated micromanipulation and micro-probing of biological samples are increasingly important methods in the today's laboratory. Microbeads as probes are the most commonly used tools in this field, although the only manipulative motion they allow is translation. We present polymerized 3D microstructures which can also be used for optical micromanipulation with more degree of freedom than microbeads.
The two-photon polymerization (TPP), based on focused fs laser beam into appropriate photopolymers is a powerful method to build structures of arbitrary complexity with submicrometer resolution. The presented tools have the advantage of being capable of twisting and rotational manipulative motion, and also that the position of biological manipulation and optical trapping is spatially separated. Different manipulative interfaces, the positioning stability and surface activation of the manipulators will be discussed.

P-735
Sequential multiplexed quantum dot labeling to profile pathway-associated protein expression of advanced human prostate cancer

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The potential application of multiplexed quantum dot labeling, MQDL, in clinical detection, prognosis and monitoring

therapeutic response has attracted high interests from bio-engineers, pathologists and cancer biologists. MQDL is superior comparing with conventional organic dye staining in its narrow emission bandwidths, wide signal dynamic ranges, high detection sensitivity, and low noise to signal ratios. However, the majority of the MQDL application has been limited to identification of specific cell type or cancer subtype and the improvement in labeling methodology. In this study, we focused on simultaneous detection and analysis of 5 proteins in the c-MET activation pathway, i.e. RANKL, VEGF, NRPLN-1, p-c-MET and Mcl-1, which are known to be associated with human prostate cancer progression and metastasis. Two experimental systems were analyzed: 1) fixed xenograft tissues from an established LTL313 castration resistance human prostate cancer or CRPC model; and 2) clinical prostate tissue specimens from localized cancer and bone metastasis. In the presentation we will report our experience in 1) the MQDL protocol optimization for the sequential reactions of individual primary antibody, the biotinylated secondary antibody and streptavidin-coated QD conjugate with nuclear DAPI staining; and 2) the multiplexed image catching, image unmixing, and subsequent per cell base quantification. For future multi-specimen analyses and validation, we will introduce a high throughput Vectra Image Analysis System.

P-736

Self assembled lipid nanostructures in aligned carbon nanotubes

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Carbon nanotubes (CNTs)[1] are already quite popular among many scientific and technological disciplines[2]. In recent years they have been targeted for biotechnological and medical applications. In this work we have investigated the nanostructural self assemblies of biological lipid molecules in presence of CNTs. Advantage of using highly aligned CNTs[3] for this purpose being the possibility of studying the interactions of lipid molecules on the macromolecular surface as well as in the confinement of aligned CNTs. We have observed various lyotropic nanostructures that are found for corresponding lipids in the bulk under dry and hydrated conditions.[4] Nanostructural studies were mainly performed using Small and wide angle X-ray scattering techniques. This work is crucial for designing the nano-micro-fluidic architectures and supported model membranes where both – functionalization of CNTs and nanostructural assembling of lipids, could be employed simultaneously.

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P-738

Neural signal recordings with a novel multisite silicon probe

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Uncovering physiological processes at the cellular level is essential in order to study complex brain mechanisms. Using multisite signal recording techniques in the extracellular space, functional connectivity between different brain areas can be revealed. A novel microfabrication process flow, based on the combination of wet chemical etching methods was developed, which yields highly reproducible and mechanically robust silicon-based multielectrode devices. The fabricated shaft of the probe is 280 μm wide, 80 μm thick, has rounded edges and ends in a yacht-bow like, sharp tip. Its unique shape provides decreased invasivity. The sensor contains 24 platinum recording sites at precisely defined locations. Murine in vivo experiments showed that the probes could easily penetrate the meninges. High quality signals, providing local field potential, multi- and single unit activities, were recorded. The interfaces between the tissue and the platinum contacts were further improved by electrochemical etching and carbon nanotube coating of the metal sites.

P-739

Protein-based integrated optical sensor device

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The integrated optical Mach-Zehnder interferometer is a highly sensitive device, considered a powerful lab-on-a-chip tool for specific detection of various chemical and biochemical reactions. Despite its advantages, there is no commercially available biosensor based on this technique. The main reason is the inherent instability of the device due to slight changes of environmental parameters. In this paper we offer a solution to this problem that enables the optimal adjustment of the working point of the sensor prior to the measurement. The key feature is a control unit made of a thin film of the light-sensitive chromoprotein bacteriorhodopsin deposited on the reference arm of the interferometer. After showing the transfer characteristics of such a device, we demonstrate its applicability to sensing of specific protein–protein interactions. We expect our method to become a rapid and cost-efficient

alternative of the commonly used measuring tools in protein research and medical diagnostics.

P-740

Nanobody-tagged polyplexes for transcriptional targeting of a lethal transgene and cancer cell killing

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MUC1 antigen is an aberrantly glycosylated glycoprotein over-expressed in tumours of epithelial origin. We have isolated an anti-DF3/Mucin1 (MUC1) single domain antibody (nanobody) and covalently linked this to the distal end of poly(ethylene glycol)$_{3500}$ (PEG_{3500}) in PEG_{3500}-25 kDa polyethylenimine (PEI) conjugates. The resultant conjugates (average of 16 PEG chains and 8.7 nanobody per PEI) successfully condensed plasmids coding a transcriptionally truncated-Bid (tBid) lethal transgene (under the control of the cancer-specific MUC1 promoter) into polyplexes of 130 nm in size and with zeta potential of close to neutrality. Polyplexes proved effective in dramatically elevating Bid/tBid expression in both MUC1 over-expressing caspase 3-deficient and caspase 3-positive tumour cell lines and induced considerable cell death. Transgene expression and concomitant cell death was exclusively promoter-specific. Our attempt not only provide a powerful proof of concept in combining nanobody-based targeting with transcriptional targeting as a safe way to deliver transgenes to specific cells, but also overcomes the known PEI-mediated cellular toxicity and minimize (or eliminate) non-targeted cell damage.

P-741

Structure-function relationships underlying anticancer activity of novel 4-thiazolidones

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Thazolidone derivatives are novel synthetic compounds possessing various biological activities. We selected three such compounds Les-3120, 3166, 3372 which passed National Cancer Institute *in vitro* tests. Annexin V/PI and DAPI staining, DNA electrophoresis in agarose gel, and Western-blot analysis using specific antibodies against 30 cellular proteins involved in apoptosis were applied to study molecular mechanisms of tumor cell death induced by these compounds. It was found that molecular targets of thiazolidones in target cells strongly depend on structure of their side groups: Les-3120 containing isatine fragment, activated caspase-8 involved in receptor-mediated apoptosis, while Les-3166 possessing benzthiazol residue, induced mitochondrial apoptosis mediated by caspase-9, and Les-3372 which has a unique chlorine atom in side chain, also led to mitochondrial apoptosis mediated by AIF (apoptosis-inducing factor). To increase anticancer potential of these molecules, *in silico* study was performed and most active groups of Les-3120 and Les-3372 were combined into one molecule. *In vitro* studies showed that such hybrid molecule called Les-3661 possessed 10 times higher anticancer potential (IC_{50}=1 μM) comparing with initial compounds.

P-742

Porous vaterite particles as drug delivery system: synthesis, encapsulation, and controlled release

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We report on the synthesis and characterization of vaterite microcontainers for controlled drug release. Moreover, we present experiments on possible release strategies of encapsulated substances via recrystallization, pH controlled, or by desorption methods.

Vaterite spherical particles were fabricated with controllable average sizes from 400±12nm till 10±1□m. We considered two ways of functionalization of the containers: encapsulation of the substances during the vaterite synthesis or their adsorption onto the prepared particles.

As model experiments, vaterite containers, encapsulating Rhodamine 6G, were imaged by two-photon microscopy, showing dye release into the aqueous medium due to recrystallization to calcite within 3 days. Differently, in ethanol only small amounts of the encapsulated markers were diffusion released after one week.

The release mechanisms can be further controlled by covering the microcontainers with additional polymer layers to increase diffusion and recrystallization time. A change of the pH from neutral to acid conditions leads to the destruction of the vaterite matrix followed by a quick release of the encapsulated materials. These flexible control mechanisms make this system an interesting candidate for pharmaceutical applications.

P-743

Label free biosensing using Grating Coupled Interferometry

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Grating Coupled Interferometry (GCI) is a label-free surface sensitive sensor introduced by us recently. It is an ideal

candidate for measuring extremely small concentrations of analytes in minute amount of samples.
The system is based on evanescent wave sensing and employs monomode thin film optical waveguides and a liquid crystal modulator for phase interrogation. In the present contribution we show some recent developments on the sensor in terms of stability, temperature control and liquid handling.

P-744
Analysis of magnetophoretic mobility of magnetic nanoparticles in magnetic field
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Magnetic nanoparticles (NP) in combination with therapeutic molecules represent one of most promising methods for targeted drug delivery. One of major current limitations of magnetic drug targeting is to achieve efficient concentration of magnetic carrier-drug complexes at the targeted sites due to poor mobility of nanoparticles in tissue structures. Interstitial delivery is hindered by microscopic extracellular matrix, which represents a major barrier for nanoparticles motilities. In order to achieve efficient magnetic drug targeting it is crucial to know particle mobility in a given in vivo environment as well as to apply magnetic field having appropriate field gradient which drags magnetic NPs.
We used gel magnetophoresis in order to measure motilities of different magnetic NPs (Co-ferrite, γ-Fe_2O_3) in agarose gel. Numerical modeling using FEM method was used to determine appropriate settings of magnets, which generate sufficient magnetic field gradient. Further, we used the numerical modeling to evaluate the magnetic force on the NPs for different geometries. We obtained that one of crucial factors which determines final mobility in tissue is formation of larger aggregates of nanoparticles under physiological conditions and interaction of nanoparticles with surrounding matrix.

O-745
Defining the forces required to gate mechanosensitive channels in mammalian sensory neurons
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Our sense of touch and mechanical pain is based on mechano-electrical transduction (MET) at the terminal endings of subsets of dorsal root ganglion (DRG) neurons innervating the skin. To quantify the stimulus strengths required to gate mechanosensitive channels in these subsets of neurons, we developed an approach using microstructured surfaces. The DRG neurons are grown on laminin-coated PDMS pillar arrays, mechanical stimuli are applied by deflecting individual pili and the deflection is monitored using light microscopy. As the pili behave as light-guides, the center of each pilus can be determined from a fit of the intensity values, allowing detection of movements of a few nanometers. The response to such stimuli is monitored using whole-cell patch-clamp. Pili deflections of 10nm can gate the rapidly adapting-current in mechanoreceptor cells, while deflections above 150nm are required for gating of slowly adapting-currents in nociceptors. Smaller stimuli are required to generate currents via pili deflection (10nm) vs neurite indentation (70-100nm), suggesting that gating occurs at the cell-substrate interface. We have also characterized the MET currents present in N2a cells which we show are modulated by the substrate to which the cells are attached.

P-746
Enhanced stimulation of Toll-like receptor 9 via immunostimulatory nanoparticles
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Among the Toll-like receptor family (TLRs), the TLR9 has been subject of intensive research because of its predominant localization in the lysosomes of immune cells and its ligand rendering it a potential candidate for immunotherapy of autoimmune diseases and cancer. Additionally, a use as an adjuvant in vaccination is aimed using synthetic CpG-oligodeoxynucleotides (CpG-ODN's). Albeit, immunostimulatory CpG-ODNs already showed promising results in animal experiments and clinical trials, several groups found that TLR9 is also expressed by tumor cells. First experiments show that activation of TLR9 displayed by cancerous cells leads to a decreased apoptosis rate and proliferation posing unpredictable threat to tumor patients exposed to CpG-ODNs. Therefore, detailed knowledge about the impact of CpG-ODNs on cancer cells is inevitable for a save use in pharmaceutics. Herein, we describe a sophisticated way to address TLR9 in cancer cells using CpG-ODN functionalized "superparamagnetic" MnO- and γ-Fe_2O_3-nanoparticles (NPs) to stimulate TLR9 in A549 cells. Analysis of impedimetric measurements revealed a cytotoxic effect of the MnO-NPs. Cells treated with immunostimulatory Fe_2O_3-NPs showed an increased micromotility as well as a higher long-term correleation of the impedance signal.

P-747
Silver sol preparation history and effectiveness of SERS diagnostics of intact red blood cells
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Surface enhanced Raman spectroscopy (SERS) technique has a number of advantages related to promising

biomedical diagnostics like high-sensitivity, single-molecule study, easy sample preparation. Furthermore, SERS allows to conduct non-invasive studies of conformations of the molecules without destruction of living cells, i.e. *in vivo* [1]. This work presents a SERS study of cytosolic hemoglobin (Hb_c) using silver nanoparticles (AgNPs). The Hb_c was isolated from cytoplasm of red blood cells taken from rat erythrocytes and diluted. AgNPs were prepared by developing Leopold and Lendl method [2]. Three types of colloids were prepared at various temperatures (25, 40 and 60 °C). The resulted AgNPs were characterized by UV-Vis-, FTIR-spectroscopy, DLS and TEM. Reduction of Ag ions leads to the formation of predominantly spherical AgNPs but also silver nanorods, faceted and aggregated AgNPs in small quantities with a surface plasmon resonance band in the range of 413 – 445 nm. For AgNPs synthesized at 25°C, for example, a bimodal size distribution was observed (about 7 and 45 nm medium sizes, respectively). SERS measurements were optimized for each type of AgNPs. It was demonstrated that AgNPs gave strong Raman enhancement from Hb_c and types of SERS spectra differ from each other.

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P-748
Conjugation of zinc oxide nanoparticles with cytochrome C

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Nano-ZnO is characterized by unique properties, low toxicity and high biocompatibility instead of a lot of others nanomaterials. For this fact nanoparticles of ZnO have great potential for applications in biosystems, for example biolabeling, biosensoring, delivery systems and others, which can be used in genetics, pathology, criminology, safety of food and many other industries. For these bioapplications are necessary surface modifications, which can made to the nanostructures to better suit their integration with biological systems, leading to such interesting properties as enhanced aqueous solubility, bio-recognition or applicability for biological systems.
For synthesis of ZnO nanoparticles in aqueous solution we used 11-mercapto-undecanoic acid (MUA) as stabilizing agent. The coating of nanoparticles with MUA could allow their solubility in the water and the binding through carboxyl groups present in its structure. We defined the optimal pH for MUA modificated nano-ZnO solubility and their ability interaction with positive charges.
We studied the optical properties of pure and surface modificated nanoparticles and their conjugates with cytochrome c and also the effect of pH on the interaction between nano-MUA and horse cytochrome c.

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P-749
Permeation through nanochannels: revealing fast kinetics

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The permeation of water soluble molecules across cell membranes is controlled by channel forming proteins and particularly the channel surface determines the selectivity. An adequate method to study properties of these channels is electrophysiology and in particular analyzing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface. As the binding of antibiotic molecules in the channels of interest is significantly weaker than that of preferentially diffusing nutrients in substrate-specific pores, the resolution of conductance measurements has to be significantly increased to be able to resolve the events in all cases. Due to the limited time resolution, fast permeation events are not visible. Here we demonstrate that miniaturization of the lipid bilayer; varying the temperature or changing the solvent may enhance the resolution. Although electrophysiology is considered as a single molecule technique, it does not provide atomic resolution. Molecular details of solute permeation can be revealed by combining electrophysiology and all atom computer modeling.

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P-750
Bio-applications of novel functionalized nanosized carriers

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Novel functionalized nanocomposites (NC) were designed and synthesized on the basis of polymeric surface-active oligoelectrolytes. The developed technology permits controlling: 1) quality and quantity of structural blocks of NC, and size unimodality; 2) branching at specific sites in polymer chain of NC; 3) providing NC with reactive chemical groups;

4) covalent conjugation of specific bio-targeting molecules. Provided bioactive elements were: a) specific anticancer drugs, antibiotics, alkaloids; b) DNA and siRNA; c) immunoglobulins and lectins; d) lipids and amino acids; e) polyethylene glycol. Fluorescent, luminescent, super-paramagnetic, or X-ray detectable compounds were also incorporated in NC to make them detectable and measurable. Biocompatible NC possessing low toxicity towards mammalian cells *in vitro* and *in vivo* (mice) were created. They were effective in delivery of: 1) drugs (doxorubicine and antibiotics) for chemotherapy *in vitro* and *in vivo*; 2) DNA for transfection of mammalian, yeast and bacterial cells; 3) protein antigens for animal immunization and specific lectins for targeting apoptotic cells. These and other approaches in application of developed NC and nanobiotechnologies are considered.

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P-751

Fluorescent nanodiamonds as cargo for siRNA delivery to Ewing sarcoma cells

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In the anti-cancer drug delivery domain, nanotechnologies are a promising tool, providing a good tissue distribution and a low toxicity. Drug delivery vehicles relying on solid nanoparticles have been proposed, among which diamond nanoparticle (size<20 nm) is a very promising candidate [1].

We have investigated the delivery of siRNA by nanodiamonds (ND) into cells in culture, in the context of the treatment of a rare child bone cancer (*Ewing* sarcoma), by such a gene therapy. siRNA was bound to NDs after NDs coating by cationic polymers, so that the interaction is strong enough to pass the cell membrane without loss of the drug and does not prevent its subsequent release.

The cellular studies showed a specific inhibition of the gene expression at the mRNA and protein level by the ND vectorized siRNA. We also uses the fluorescence of color center created in the nanodiamonds [2] to monitor the release of fluorescently-labeled siRNA in the intracellular medium. This technique brings a quantitative insight in the efficiency of siRNA to stop cell proliferation.

Considering the success of the cell model we recently started the drug delivery in tumor xenografted on nude mice.

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O-752

Multiscale pattern fabrication for life-science applications

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The combination of unconventional fabrication technology and biomaterials allows both to realize state-of-the-art devices with highly controlled lateral features and performances and to study the main properties of the biomolecules themselves by operating at a scale level comparable with the one crucial for their activity. Soft lithography and microfluidic devices offer a tool-box both to study biomolecules under highly confined environments [1] and to fabricate in an easy way topographic features with locally controlled mechanical and chemical surface properties, thus leading to a finer control of the interplay of mechanics and chemistry. I will present an application of this technology to the control of cell fate that is becoming a key issue in regenerative medicine in the perspective of generating novel artificial tissues.

Patterns of Extracellular Matrix (ECM) proteins have been fabricated, by a modified Lithographically Controlled Wetting (LCW), on the highly antifouling surface of Teflon-AF to guide the adhesion, growth and differentiation of neural cells (SHSY5Y, 1321N1, NE-4C) achieving an extremely accurate guidance [2]. Local surface topography is also known to influence the cell fate [3], thus, integrating this parameter in the substrate fabrication could increase the complexity of the signals supplied to the cells. In this perspective we have developed a novel fabrication technique, named Lithographically controlled Etching (LCE), allowing, in one step, to engrave and to functionalize the substrate surface over different lengthscales and with different functionalities.

I will conclude showing how we have been developing ultrathin film organic field effect transistors (OFETs) as label-free biological transducers and sensors of biological systems. OFETs are low dimensional devices where ordered conjugated molecules act as charge transport material. Unconventional patterning techniques and microfluidics have been adapted to proteins and nucleic acids to dose the molecules on the OFET channel with a high control of the concentration. In another set of experiments, we have also been addressing the signalling from neural cells and networks grown on pentacene ultra-thin film transistosr [3,4].

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P-753
Alterations of immune response of lymphocytes exposed to PLGA and TiO2 nanoparticles *in vitro*
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Advances in nanotechnology are beginning to exert a significant impact in medicine. Increasing use of nanomaterials in treatment of diseases has raised concerns about their potential risks to human health. In our study, the effect of poly(lactic-co-glycolic acid) (PLGA) and titanium dioxide (TiO_2) nanoparticles (NPs) on function of B- and T- lymphocytes was investigated in vitro.
Human blood cultures were treated with PLGA and TiO_2 NPs in concentrations: 0.12; 3 and 75 $\mu g/cm^2$ for 72h. Lymphocyte transformation assay was used to assess the effect of NPs on lymphocyte function. Lymphocytes were stimulated with mitogens: concanavalin A, phytohaemmagglutinin (T-cell response) and pokeweed mitogen (B-cell response).
Our findings indicate immunomodulatory effect of PLGA NPs. Proliferative response of T- and B-lymphocytes exposed in vitro to the highest dose of PLGA for 72h was suppressed significantly ($p<0.01$, $p<0.05$). On the other hand, we observed stimulative effect of exposure to middle dose of PLGA NPs on B-lymphocyte proliferation ($p<0.05$). No alteration was found in lymphocyte proliferation treated in vitro with TiO_2 NPs for 72h.
In conclusion, proliferation of lymphocytes in vitro might be one of the relevant tests for evaluation of NPs immunotoxicity.
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P-754
Using amino functionalized ORMOSIL nanoparticles as a non-viral DNA vector
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Silica nanoparticles are stable aqueous suspension of condensed siloxane nanocomposites, having an average diameter between 10 and 100 nm. Particles containing organic functional groups on their surface are called Organically Modified Silica Nanoparticles (ORMOSIL). Due to the various chemical and physical properties of the surface groups, ORMOSIL nanoparticles may have an enormous variety of biological applications, such as in vivo bioimaging, non-viral gene delivery or targeted drug delivery. Our aim was to synthesize both void and fluorescent dye doped amino functionalized ORMOSIL nanoparticles through the microemulsion method and use them for gene delivery. The obtained nanoparticles have been characterized by transmission electron microscopy and dynamic light scattering. Furthermore, the nanoparticles have been investigated to exploit their transfection efficiency and the possible toxicity caused by surfactants used in the synthesis. The transfection efficiency was tested on various cell cultures. Our further aim is the in vivo transfection of salivary glands using ORMOSIL nanoparticles. Our work has shown that the nanomedicine approach, with nanoparticles acting as a DNA-delivery tool is a promising direction for targeted gene therapy.

Bioengineering & biotechnology

P-755
***In vivo* amperoetric cells for detection of fast diffusing, physiologically important small molecules**
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H_2S is a naturally occurring gas that is toxic in high concentration. It exists also in different tissues of living animals sometimes in concentrations as high as 20 μM. It is generally accepted, that H_2S has important roles in modulating different, physiologically important biochemical processes similarly to other, fast diffusing molecules like NO, CO and H_2O_2.
For investigation of the physiological effects of these species their local concentration in the studied biological media is important to know. This means methods needed for measuring the instantaneous concentration with high spatial resolution in living tissues without major invasion. Electrometric micro, and ultramicro sensors are often gain application in experimental life sciences for measurement of local ion concentration or following neurotransmitter species in vivo measurements.
In our work efforts are being carried out to improve the applicability of selective electrometric sensors in life science experiments. As a result of these work an improved H_2S measuring cell and improved electrode and method was developed for measurement of electroactive small molecules like NO or H_2O_2.
In the poster to be presented the structure, the working principles and the performances of the different sensors mentioned will be described.

O-756

Engineered bacteriorhodopsin: a molecular scale conductance photoswitch

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Bacteriorhodopsin (BR) is the only protein in the purple membrane of the halophilic organism *Halobacterium salinarium*. It is a light-driven proton pump converting light into a transmembrane proton gradient through isomerization of the covently bound retinal chromophore. Its stability, as well as its photoactivity in dried films, has made BR an attractive material for biomolecular devices. Such studies, however, have used BR within the membrane, on relatively large surfaces. Here, conducting-probe atomic force microscopy (C-AFM) analysis was performed after isolating the protein from its native membrane environment while keeping its basic trimeric structure, and demonstrated that the molecular conductance of BR can be reversibly photoswitched with predictable wavelength sensitivity. Intimate and robust coupling to gold electrodes was achieved by using a strategically engineered cysteine mutant located on the intracellular side of the protein which, combined with a 75% delipidation, generated protein trimers homogenously orientated on the surface. C-AFM proximal probe analysis showed a reproducible 3 fold drop of BR mean resistance over $\sim$5 cycles of interspersed illuminations at the same gold-bR-gold junction when λ>495 nm, while no shift was observed with other wavelengths.

P-757

Capture of circulating tumor cells with a highly efficient nanostructured silicon substrates with integrated chaotic micromixers

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A new-generation technology for cancer diagnosis, led by H.R. Tseng at UCLA and L.W.K. Chung at Cedars-Sinai Medical Center, was developed for improved harvesting of circulating tumor cells from the blood of patients with prostate cancer. This technology has the capability to enrich circulating tumor cells (CTCs) with high efficiency by integrating an antibody-coated Silicon nanopillar (SiNP) substrate with an overlaid polydimethylsiloxane (PDMS) microfluidic chaotic mixer (Angew. Chem., doi:10.1002/ange.2010005853, 2011). This core technology shows significantly improved sensitivity in detecting rare CTCs from whole blood, thus provides an alternative for monitoring cancer progression. By assembling a capture-agent-coated nanostructured substrate with a microfluidic chaotic mixer, this integrated microchip can be applied to isolate CTCs from whole blood with superb efficiency. Ultimately, the application of this approach will open up opportunities for early detection of cancer metastasis and for isolation of rare populations of cells that cannot feasibly be done using existing technologies. This technology helped to find a needle in a haystack and will open up the opportunity for single cell genomic and epigenetic sequencing and gene expression profiling. Results from further development of this technology will assist the physicians in follow-up patients and testing vigorously the concept of personalized oncology with individualized therapy. This novel technology has recently been reviewed and highlighted by Nature Medicine (17:266: March 2011).

O-758

Membrane biohybrid systems for tissue and organ engineering

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The growing crisis in organ transplantation and the aging population have driven a search for new and alternative therapies by using advanced bioengineering methods. The formation of organized and functional tissues is a very complex task: the cellular environment requires suitable physiological conditions that, presently, can be achieved and maintained by using properly-designed bioreactors reproducing all specific functions and bioactive factors that assure viability/regeneration of cells cultured in an appropriate scaffold. The creation of biomimetic environment requires the use of biomaterials such as membranes with specific physico-chemical, morphological and transport properties on the basis of the targeted tissue or organ. Tailor-made membranes (organic, functionalized with specific biomolecules, in hollow-fiber configuration), designed and operated according to well-defined engineering criteria are able to sustain specific biotransformations, to provide adequate transport of oxygen, nutrients and catabolites throughout the cellular compartment, and to supply appropriate biomechanical stimuli of the developing tissue. In this talk the author will show the development of membrane engineered constructs focusing on liver and neuronal systems. The role of membrane surface and transport properties in providing instructive signals to the cells for the guiding of proliferation and differentiation will be discussed. Membrane bioreactors, which through the fluid dynamics modulation may simulate the in vivo complex physiological environment ensuring an adequate mass transfer of nutrients and metabolites and the molecular and mechanical regulatory signals, will be presented.

P-759

An original microfabricated cell culture substrate for cell-based assays

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Here we present a novel but simple system for cell-based assays enabling simultaneous testing of multiple samples on a same tissue without cross-contamination between neighbouring assays, as well as sequenced or repeated assays at the same tissue location. The principle of this method lies in the spatially-controlled diffusion of test compounds through a porous matrix to the target cells. A simple microfabrication technology was used to define areas where diffusion processes are allowed or inhibited. We performed proof-of-principle experiments on Madin-Darby canine ***kidney*** (MDCK) epithelial cells using Hoechst nuclear staining and calcein-AM cell viability assay. Fluorescent staining superimposed properly on membrane pattern with a dose-dependent response, indicating that both compounds specifically and selectively diffused to the target cells. MDCK cells similarly treated with cytochalasin B showed their actin network rapidly altered, thus demonstrating the suitability of this system for drug screening applications. Such a well-less cell-based screening system enabling multiple compounds testing on a same tissue and requiring very small volumes of test samples appears interesting for studying potential combined effects of different biochemicals applied separately or sequentially.

P-760

Subpicosecond all-optical switching by the protein bacteriorhodopsin

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It is generally believed that all-optical data processing is the most promising direction to achieve serious improvements both in capacity and speed of Internet data traffic. One of the bottlenecks of the state-of-the-art photonic integration technology is to find the proper nonlinear optical (NLO) materials that are supposed to serve as cladding media in waveguide-based integrated optical circuits performing light-controlled active functions. Recently, the unique chromoprotein bacteriorhodopsin (bR) has been proposed to be used as an active, programmable NLO material in all-optical integrated circuits. In integrated optical applications of bR, its light-induced refractive index change is utilized. In this paper we exploit the refractive index changes of a dried bR film accompanying the ultrafast transitions to intermediates I and K, which allows even sub-ps switching, leading beyond Tbit/s communication rate. In the experiments direct pulses of a femtosecond laser system at 800 nm were used along with synchronized ultrafast laser pulses at 530 nm. We believe that the results may be the basis for the future realization of a protein-based integrated optical device, and represent the first steps to a conceptual paradigm change in optical communication technologies.

P-761

SEREX-derived antigens for detection of breast cancer serum autoantibody profile

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Molecular alterations following malignant transformation of cells stimulate systemic immune response in human body. This response can be avaluated by detection of circulated autoantibodies directed to corresponding tumor-associated antigens. Last years, such autoantibodies attract an increasing attention of researchers as potential cancer biomarkers. Since the sera of cancer patients typically contain a unique set of antibodies that reflect the tumor-associated antigens expressed in a particular malignant tissue, diagnosing and predicting the outcome of disease such as breast cancer based on serum autoantibody profiling is an attractive concept.

To create a representative panel of antigens for detecting of breast cancer autoantibody profile we selected 18 breast cancer associated antigens. These antigens were identified by screening of tumor cDNA libraries with autologous sera using SEREX (*SE*rological investigation of *R*ecombinantly *EX*pressed clones) approach. All antigens were cloned, expressed, purified in bacteria and tested with sera of breast cancer patients and healthy donors in large-scale allogenic screening using ELISA.

The utility of selected tumor associated antigens for detecting of autoantibody profile in different types of breast cancer was evaluated.

O-762

Organ printing: the key to eternal life?

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We introduce a novel automated rapid prototyping method (organ printing) that allows engineering fully biological three-dimensional custom-shaped tissue and organ modules. In this technology bio-ink units (multicellular aggregates) composed of single or several cell types together with supporting material are delivered by special printers. Printing of the bio-ink units

(controlled by architectural software) is carried out according to a design template, consistent with the geometry and composition of the desired organ module. Structure formation occurs by the post-printing fusion of the discrete bio-ink units. When the bio-ink units contain more than one cell type, fusion is accompanied by sorting of the cells into the physiologically relevant pattern. Thus structure formation takes place through self-assembly processes akin to those utilized in early embryonic morphogenesis. We demonstrate the technology by detailing the construction of vascular and nerve grafts. Spherical and cylindrical bio-ink units have been employed to build fully biological linear and branching vascular tubular conduits and multiluminal nerve grafts. Upon perfusion in a bioreactor the constructs achieved desirable biomechanical and biochemical properties that allowed implantation into animal models. Our results show that the printing of conveniently prepared cellular units is feasible and may represent a promising tissue and organ engineering technology.

P-765

Femtosecond laser assisted microsurgery of mammalian cells for medical and biotechnology applications

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Femtosecond lasers have become important tools for non-contact microprocessing of biological specimens. Due to the short pulse length and intensity-dependent nature of the multiphoton ionization process, fs-laser pulses affect only a small volume of a treated cell, providing a high degree of spatial localization. We employed fs-laser to address topical bioengineering and biomedical problems such as cell fusion and embryo biopsy respectively. A tightly focused laser beam (Cr:F seed oscillator and a regenerative amplifier, 620nm, 100 fs, 10 Hz) was used for a fusion of blastomeres of two-cell mouse embryos and for a polar body (PB) biopsy. In order to fuse blastomeres the contact border of cells was perforated by a single laser pulse. The fusion process usually completed within $\sim$60 min. In order to perform a noncontact laser based PB biopsy we initially drilled an opening in the zona pellucida with a set of laser pulses, and then extracted the PB out of zygote by means of optical tweezer (cw laser, 1064 nm). The energy of laser pulses was thoroughly optimized to prevent cell damage and increase the fusion and biopsy rates. The proposed techniques demonstrate high efficiency and selectivity and show a great potential for using fs lasers as a microsurgical tool.

P-766

New insights into mechanisms of electric field mediated gene delivery

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Gene electrotransfer is widely used for transfer of genetic material in biological cells by local application of electric pulses and is currently the most promising non-viral delivery method for gene therapy for a series of diseases as well as for DNA vaccination. Current description of the process defines several steps: electropermeabilization, DNA-membrane interaction, translocation, trafficking to nucleus and into nucleus. But the mechanisms of electrotransfer are still not fully understood.

We present results of the systematic in vitro analysis using pEGFP of all steps involved in electrotransfection from electropermeabilization, analysis of different pulsing protocols, theoretical analysis of plasmid mobility to visualization of the processes of DNA-membrane interaction. We demonstrate that in order to translate in vitro results to tissue level sub-optimal plasmid concentrations have to be used. Furthermore, sofar the method of DNA entry into cytoplasm was only speculated. Our results suggest that it is crucial that first, membrane is electropermeabilized, then sufficient electrophoretic force is crucial for insertion of DNA into destabilized lipid bilayer followed by DNA translocation into cytoplasm via a slow process. Efficiency of electrotransfer depends also on the stage of of cell culture – cells in dividing phase are easer to electrotransfect.

P-767

Gentamicin interaction with B16F10 cell membrane studied by dielectrophoresis

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Dielectrophoresis (DEP) is the translational motion of polarizable particles due to an electric field gradient. Positive-DEP and negative-DEP correspond to particle movement forward or backward the region of high field intensity, respectively. Our study reveals some of the cell membrane modifications induced by Gentamicin (Gt), as they are reflected in the ***crossover frequency*** $\mathbf{f_{CO}}$ of B16F10 murine cells incubated with Gt for different concentrations and durations. $\mathbf{f_{CO}}$ is the AC frequency when cells turn from positive-DEP to negative-DEP. Gentamicin is a positively charged aminoglycosidic antibiotic, with concentration-dependent killing action; it is widely used because of its low cost and reliable bactericidal activity. Gt drawbacks consist in high toxicity for renal and hearing cells; the molecular mechanisms of this toxicity are still unclear. For low external medium conductivities ($\approx$ 0.0012 S/m), $\mathbf{f_{CO}}$ of control and Gt-cells was found to range from 3 to 10kHz. $\mathbf{f_{CO}}$ shifts to higher frequencies with the increase of Gt concentration and incubation time. Cells dielectrophoretic behavior is discussed using the cell single-shell based model.

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P-768

PEGylation increases mobility of chitosan-DNA delivery systems in artificial extracellular matrices

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Extracellular matrix (ECM) is a major obstacle for succesful delivery of genes. Chitosan is a versatile and biocompatible polysaccharide derived from chitin and is a promising gene carrier. Chitosan-DNA interactions, and hence DNA polyplexation and release can be controlled through chitosan de-acetylation degree, molecular weight and functionalization of chitosan cationic groups. Grafting of poly(ethylene glycol) PEG to gene delivery vectors increases circulation time of gene delivery systems in blood vessels and reduces polyplexes charge. Diffusion and unpacking of PEGylated and non-PEGylated chitosan-DNA polyplexes through articial ECMs based on collagen and collagen-hyaluronic acid (HA) gels were compared using fluorescence correlation microscopy, confocal microscopy and colocalization analysis. Non-PEGylated polyplexes were immobilized in the gels whereas PEGylated polyplexes were diffusing. The smaller charge of PEGylated polyplexes seems to decrease interactions between polyplexes and ECM components. Furthermore, HA might also screen collagen fibers-PEGylated polyplexes interactions. PEGylated polyplexes also showed a higher degree of unpacking in gels, probably due to a looser compaction of DNA by PEGylated chitosan compared to non-PEGylated chitosan.

O-769

Continuous droplet interface crossing encapsulation for high through-put monodisperse vesicle design

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Fabrication of vesicles, a close membrane made of an amphiphile bilayer, has great potentiality for encapsulation and controlled release in chemical, food or biomedical industries but also from a more fundamental point of view for the design of biomimetic objects.
Methods based on lipid film hydratation[1], inverse emulsion techniques[2] and more recently microfluidic techniques such as double emulsion[3] or jetting[4] method are limited either by a low yield, a low reproducibility, a poor control on the size, or by the presence of remaining solvent or defects.
We propose a fast and robust method[5] easy to implement: Continuous droplet interface crossing encapsulation (cDICE), that allows the production of defect-free vesicles at high-yield with a control in size and content. The vesicles have controlled bilayer composition with a polydispersity in size lower than 11%. we have shown that solutions as diverse as actin, cells, micrometric colloids, protein and high ionic strength solutions can easily be encapsulated using this process. By adjusting the parameters of our set-up, we are able to produce vesicles in the range 4-100 μm in diameter, stable for weeks. We believe this method open new perspectives for the design of biomimetic systems and even artificial tissues.

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O-770

Acceleration neuronal precursors differentiation induced by substrate nanotopography

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Embryonic stem (ES) cell differentiation in specific cell lineage is still a major challenge in regenerative medicine. Differentiation is usually achieved by using biochemical factors (BF) which concentration and sides effects are not completely understood. Therefore, we produced patterns in polydimethylsiloxane (PDMS) consisting of groove and pillar arrays of sub-micrometric lateral resolution as substrates for cell cultures. We analyzed the effect of different nanostructures on differentiation of ES-derived neuronal precursors into neuronal lineage without adding biochemical factors. Neuronal precursors adhere on PDMS more effectively than on glass coverslips but the elastomeric material itself doesn't enhance neuronal differentiation. Nano-pillars increase both precursors differentiation and survival with respect to grooves. We demonstrated that neuronal yield was enhanced by increasing pillars height from 35 to 400 nm. On higher pillar neuronal differentiation reaches ~80% 96 hours after plating and the largest differentiation enhancement of pillars over flat PDMS was observed during the first 6 hours of culture. We conclude that PDMS nanopillars accelerate and increase neuronal differentiation.

O-771

A polymerizable GFP variant

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Flagellin is the subunit protein of bacterial flagellar filaments. It has the ability to polymerize in vitro into long filaments

under appropriate conditions. The extremely variable D3 domain of flagellin subunits, comprising residues 190–283, protrudes at the outer surface of flagellar filaments. The D3 domain has no significant role in the construction of the filament structure. Thus, replacement of D3 may offer a promising approach for insertion of heterologous proteins or domains without disturbing the self-assembly of flagellin subunits. Our work aims at the construction of flagellin-based fusion proteins which preserve the polymerization ability of flagellin and maintain the functional properties of the fusion partner as well. In this work a fusion construct of flagellin and the superfolder mutant of green fluorescent protein (GFP) was created. The obtained GFP variant was highly fluorescent and capable of forming filamentous assemblies. Our results imply that other proteins (enzymes, binding domains etc.) can also be endowed by polymerization ability in a similar way. This approach opens up the way for construction of multifunctional filamentous nanostructures.

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P-772

Gene delivery targeted to the TAG72 overexpressing tumour cells using an nanobody conjugated polyethyleneglycol-modified polyamidoamine dendrimer

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Keywords: Polyamidoamine (PAMAM) Dendrimer, hetrobifunctional PEG, anti-TAG72 nanobody.

Generation 5 Polyamidoamine (PAMAM) Dendrimer has been shown to be highly efficient nonviral carriers in gene delivery. However, their toxicity limits their applications. In this study, to improve their characteristics as gene delivery carriers, G5 PAMAM Dendrimer was modified with anti-TAG72 nanobody through hetrobifunctional PEG, then complexed with t-Bid coding pDNA, yielding PAMAM-PEG-Anti-TAG72 nanobody/pDNA nanoparticles (NPs). Nuclear magnetic resonance (NMR) spectroscopy, zeta sizing and gel retardation assay results provided evidence that the nanovector was successfully constructed. The transfection efficiency of vector/pDNA complexes were evaluated in vitro. Real time PCR results also demonstrated that Anti-TAG72 nanobody modified NPs are more efficient in t-Bid killer gene expressing in colon cancer cell line than the unmodified NPs. In conclusion, PAMAM–PEG–anti-TAG72 nanobody showed great potential to be applied in designing tumour-targeting gene delivery system.

P-773

Macromolecular crowders and stem cell differentiation

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Macromolecular crowding (MMC) is a biophysical tool which has been used extensively to enhance chemical reactions and biological processes by means of the excluded volume effect (EVE). The *in vivo* stem cell microenvironment contains macromolecules which are crucial for stem cell self-renewal and cell fate determination. In order to mimic this physiological microenvironment, crowders are included in cell culture medium. We have observed that the *ex vivo* differentiation of human mesenchymal stem cells (hMSCs) into the adipogenic lineage is significantly amplified when a crowder mixture comprising Ficoll 70 and Ficoll 400 is added to the culture medium. Stem cell differentiation is modulated by soluble chemical substances as well as interactions between cells and the extracellular matrix (ECM), and both these external influences may be affected by MMC. Measurements we have performed by fluorescence correlation spectroscopy (FCS) show that Ficoll additives cause anomalous subdiffusion within a crowder concentration range of 0 to 300 mg/ml. The diffusion of fluorophore-labelled molecules in artificial lipid bilayers and membranes of living cells is not changed by crowders, suggesting that these crowders do not directly alter membrane properties and cell surface signalling. However, we have data to suggest that crowders increase actin polymerization reaction rates *in vitro*. We have also observed that crowders are taken up by stem cells and that they localize to specific compartments. Based upon our observations, we hypothesize that crowders can influence stem cell differentiation by influencing molecular kinetics.

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Inspecting laccase action through radical generation

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Lignocellulose-based composites are becoming extremely important and perspective sustainable and renewable natural materials. Fibre modification enhancing their existing properties can be obtained to broaden the application areas. In response to shortcomings of traditional chemical and physical methods, enzymes and chemo-enzymatic methods have emerged as eco-friendly catalysts working under mild conditions and enable tailoring of the material surface properties by substrate specificity and regional selectivity. Recently,

binding of different functional molecules to lignin-rich fibres by using an oxidative enzyme (e.g. laccase) has been reported leading to their functionalisation through free radical reactions. By the application of electron paramagnetic resonance spectroscopy (EPR) laccase action was inspected. Consumption of substrates was investigated and their polymerization traced. Stable radical intermediates were detected with EPR when substrate molecules were in contact with active enzymes. Secondly, oxidation of mediators like nitroxides was determined via EPR spectroscopy of stable water-soluble nitroxide radicals. Finally, the generation of short-lived radicals as well as their reduction was measured via EPR spin trapping using DMPO as sensitive water soluble spin trap.

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The effect of ovary hormone stimulation on mouse oocyte and early embryo electric conductivity

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Mammalian ovary hormone stimulation (OHS) is known to be an inalienable stage of reproductive biotechnology as well as human infertility treatment. The basic aim of the OHS is to receive a stock of valuable oocytes and early embryos for subsequent utilization in the reproductive technology, experimental work et al. However, it is known that OHS itself affects the character of ovulation and oocyte quality, which in its turn affects the development of embryos and even has distant consequences. The wideness of cell parameters and appropriate methods for investigation of gamete/embryo quality are very important. The aim of this study is determination of specific electric conductivity of mouse oocytes and early embryos which have been received after OHS in comparison with the ones that have been received in natural animal sex cycle. Using techniques of electroporation the dependence of specific electric conductivity of mouse oocytes, zygotes, 2-cell and 8-cell embryos on the external electric field intensity has been studied. It is shown that the whole pool of oocytes that were obtained in the result of OHS consists of two groups of oocytes that don't differ from each other morphologically, but differ by their electric parameters and resistance to electric breakdown. At the zygote stage, dividing of embryos into two groups is preserved, but is less expressed. At the stage of 2-cell and 8-cell dividing of embryos into two groups on their electric conductivity disappeared but certain scattering of the parameters due to individual embryo peculiarities is observed. The obtained data show that OHS may lead to latent changes of oocyte state that in their turn affect embryo quality.

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FTIR spectroscopic study of biopolyester synthesis traits in the bacterium *Azospirillum brasilense*

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Many microbes synthesize and accumulate granules of polyhydroxyalkanoates (PHA, biodegradable storage materials alternative to traditional plastics), which help them survive under stresses. In particular, the plant-growth-promoting rhizobacterium *Azospirillum brasilense*, that is under investigation worldwide owing to its agricultural and biotechnological significance, can produce poly-3-hydroxybutyrate (PHB) [1]. In our work, PHB synthesis in *A. brasilense* cells was studied under various stresses using diffuse reflectance FTIR spectroscopy. PHB in cells was determined from the band intensity ratio of the polyester ν(C=O) at $\sim$1740 cm^{-1} to that of cell proteins (amide II band at $\sim$1550 cm^{-1}), showing *A. brasilense* to be able to produce PHB up to over 60% of cells' dry weight. Stresses induced PHB accumulation, enhancing IR absorption in PHB specific regions. Analysis of a few structure-sensitive PHB vibration bands revealed changes in the degree of intracellular PHB crystallinity (related to its enzymatic digestion rate) at different stages of bacterial growth, reflecting a novel trait of the bacterial adaptability to an enhancing stress, which is of great importance to agricultural biotechnology.

[1] Kamnev AA, Tugarova AV, Antonyuk LP et al. (2006) *Anal Chim Acta* 573-574: 445-452.

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Development of a xylanase A variant capable of forming filamentous assemblies

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The aim of this work is to furnish enzymes with polymerization ability by creating fusion constructs with the polymerizable protein, flagellin, the main component of bacterial flagellar filaments. The D3 domain of flagellin, exposed on the surface of flagellar filaments, is formed by the hypervariable central portion of the polypeptide chain. D3 is not essential for filament formation. The concept in this project is to replace the D3 domain with suitable monomeric enzymes without adversely affecting polymerization ability, and to assemble these chimeric flagellins into tubular

nanostructures. To test the feasibility of this approach, xylanase A (XynA) from *B. subtilis* was chosen as a model enzyme for insertion. With the help of genetic engineering, a fusion construct was created in which the D3 domain was replaced by XynA. The FliC(XynA) chimera exhibited catalytic activity as well as polymerization ability. These results demonstrate that polymerization ability can be introduced into various proteins, and building blocks for rationally designed assembly of filamentous nanostructures can be created (Table 1).

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