EBSA 2015

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EBSA Biophysics in Europe

10th European Biophysics Congress

July 18 to 22, 2015 – Dresden, Germany
International Congress Center Dresden – ICCD

www.ebsa2015.org

Published by

EBSA 2015 Congress Office
c/o Max Planck Institute for Biophysical Chemistry
Department of Theoretical and Computational Biophysics
Am Fassberg 11
37077 Göttingen, Germany

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July 03, 2015

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— *Late Abstracts* —

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L-786

Investigating allosteric regulation through enhanced sampling simulations

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Allosteric regulation plays such a fundamental role in biology to be described by Jacques Monod as 'the second secret of life'. In signalling proteins such as protein kinases, ligand binding to allosteric sites have been shown to be able to up- or down-regulate the catalytic activity. Understanding the molecular mechanisms underlying the observed allosteric effects is of great importance for the rational design of novel biologically active allosteric regulators. One major challenge is the accurate description of the conformational landscape prior to and upon the binding of the allosteric regulator. To this aim we have developed and applied metadynamics-based enhanced sampling methods, such as parallel-tempering metadynamics and the "well-tempered ensemble". Here we show how we these methods were successfully used to rationalise the mode of action of two allosteric kinase regulators and to design more potent derivatives.

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Abstracts

– 1. Protein Structure and Function –

L-787

A SAXS-based analysis of membrane-solved CFTR under different pharmacological conditions

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Mutations of CFTR gene cause cystic fibrosis. Small-angle x-ray scattering (SAXS) technique was used to investigate the conformation of CFTR protein in microsomal membranes extracted from NIH/3T3 cells permanently transfected with wild type (WT) CFTR and with CFTR carrying the delF508 mutation. The electronic density profile of the membranes was calculated assuming the lipid bilayer electronic density to be composed by a series of Gaussian shells. Membranes in the microsome vesicles result oriented in the outside-out conformation. Phosphorylation of CFTR does not change the electronic density profile of the microsome vesicles, while dephosphorylation produces a modification in the inner side of the profile. We conclude that CFTR in microsomes is mostly phosphorylated. The electronic density profile of delF508-CFTR is different from WT, suggesting a different assemblage of the protein in the membranes. Low temperature treatment of cells rescues the delF508-CFTR protein, resulting in a conformation that resembles the profile of microsomes containing the WT-CFTR. Treatment with the corrector VX-809 modifies the electronic profile of delF508-CFTR, but does not recover completely the WT conformation.
Work supported by Fondazione per la Ricerca sulla Fibrosi Cistica, grant FFC#4/2012

L-788

Altering in the structure of neuropeptide Y during the binding to the Y1R investigated by NMR

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GPCRs are involved in most physiological processes and have become of high interest for the research community as well as for the pharmaceutical industry. Unfortunately, molecular characterisation of these large transmembrane proteins is difficult. Solution nuclear magnetic resonance (NMR) spectroscopy offers the opportunity to study structural and dynamical aspects in the interaction of ligand and receptor. Using different NMR experiments we try to understand how the neuropeptide Y (NPY) bound to the neuropeptide Y receptor type 1 (Y1R). Therefore, several differently $^{15}N/^{13}C$-labelled NPY variants were synthesized by solid phase peptide synthesis and studied bound to the receptor by NMR. The Y1R was produced recombinantly in *E.coli*, refolded and incorporated in DMPC/DHPC bicelles. We determined several changes in the NPY backbone bound to Y1R in comparison to the not bound state via recording different $^{1}H/^{15}N$ HSQC spectra by solution NMR. Finally, our $^{13}C/^{13}C$ correlation spectra recorded by solid state NMR indicate a change in the secondary structure of NPY bound to the receptor. Taken together, the binding of NPY to the Y1R is connected with a considerable altering in the structure of the ligand.

L-789

The role of transmembrane domains in the structure and function of carnitine palmitoyltransferase 1

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Carnitine Palmitoyltransferase 1 (CPT1) is an outer mitochondrial membrane protein that plays an important role in the β-oxidation of long chain fatty acids by regulating their entry into the mitochondrial matrix. This regulatory property is due to its inhibition by malonyl-CoA. Three isoforms have been identified: CPT1A (which is abundant in the liver); CPT1B (which occurs in heart and skeletal muscles and other highly oxidative tissues e.g. brown adipose) and a brain isoform (CPT1C). CPT1A and CPT1B have a high degree of similarity in primary sequence, however CPT1B has 30-100 fold greater sensitivity to malonyl-CoA. CPT1A and CPT1B are both predicted to have two transmembrane (TM) domains which are thought to interact both inter- and intramolecularly, and to sense the membrane environment in which they occur. These interactions are thought to be important for the structure and the function of CPT1. The potential for modulating the function of the three different CPT1 isoforms is of therapeutic interest for the treatment of many metabolic disorders, most notably diabetes. The main focus of my project is to elucidate the role of the transmembrane domains in the structure and function of CPT1A and CPT1B using biological and biophysical methods.

L-790

Small angle X-Ray scattering for structural analysis of biological macromolecules in solution

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Small angle X-ray scattering (SAXS) is a powerful technique to unravel the structure and interactions of biological macromolecules such as proteins and nucleic acids with a size of kDa up to GDa. In contrast to X-ray crystallography, the molecules are studied in solution allowing for time-resolved investigations and measurements under a broad range of solution conditions. Thereby, only small sample volumes (10-30μl) and moderate protein concentrations ($\sim$ 1mg/ml) are required.SAXS experiments are routinely performed at state of-the art 3rd generation synchrotron X-ray sources. As a complimentary approach we present an in-house setup for SAXS measurements. As a proof-of-concept, we collected SAXS data on DNA samples, proteins and micelles. Beside the determination of global structural parameters from the in-house data, we could perform *ab-initio* shape reconstruction of low-resolution (3-1nm) 3D models of the biomolecules.Moreover, we are studying the large blood glycoprotein von Willebrand factor (vWF), which plays an important role in hemostasis and thrombosis. Recently, it was found that different hemodynamic conditions influence the static structure of VWF. Thus, SAXS measurements should provide further insight into solution-dependent structural dynamics of vWF.

Abstracts

– 1. Protein Structure and Function –

L-791

Biophysical and structural characterization of BARD1BRCT domain and cancer predisposing mutants

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BRCT domains are evolutionarily conserved phosphopeptide binding motif and have a wide role in DNA damage repair (DDR) and cell cycle regulation. BARD1BRCT employs tumor suppressor function by interacting with DDR proteins in a phosphodependent manner. Five cancer predisposing mutations C645R, R658C, V695L, I738V and S761N are reported in BARD1BRCT domain. To understand the effect of mutation on the structure and function, purified BARD1BRCT wild type and the mutant proteins were subjected to chemical crosslinking assay, FPLC, DLS and mass spectrometric analysis to detect the changes in molecular behavior and identity of proteins. Fluorescence, CD spectroscopy and limited proteolysis concluded that WT and mutants have well folded structural conformation. However, thermal and chemical denaturation studies reveal drastic decrease in the thermodynamic stability of V695L, S761N, R658C and I738V mutants as compared to wild type and all proteins unfold via intermediate formation. MD simulation studies on wild type and mutant protein structures indicate that the mutants have lost structural integrity compared to the wild type protein. Comparative study of wild type and mutants will be helpful in understanding the role of BARD1BRCT in DDR, cell cycle regulation and tumorigenesis.

L-792

MutS/MutL crystal structure reveals that the MutS sliding clamp loads MutL onto DNA

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To avoid mutations in the genome, DNA replication is generally followed by DNA mismatch repair (MMR). MMR starts when a MutS homolog recognizes a mismatch and undergoes an ATP-dependent transformation to an elusive sliding clamp state. How this transient state promotes MutL homolog recruitment and activation of repair is unclear. Here we present a crystal structure of the MutS/MutL complex using a site-specifically crosslinked complex and examine how large conformational changes lead to activation of MutL. The structure captures MutS in the sliding clamp conformation. Our work explains how the sliding clamp promotes loading of MutL onto DNA, to activate downstream effectors. We thus elucidate a crucial mechanism that ensures that MMR is initiated only after detection of a DNA mismatch.

L-793

Interleukin-1 β and fragment A effects on the activity of eukaryotic elongation factor 2 (eEF2)

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Eukaryotic elongation factor 2 (eEF2) promotes ribosomal translocation in polypeptide chain elongation. (ADP)-ribosylation is a post-translational modification reaction that catalyzes the transfer of ADP-ribose group to eEF2 and this causes the inhibition of protein synthesis. eEF2 can be ADP-ribosylated in the lack of diphtheria toxin (DTx) and this is known as endogenous ADP-ribosylation. DTx is a well-characterized bacterial protein toxin. Fragment A (FA) of DTx in the cell leads to catalyze the ADP-ribosylation of eEF2 and that causes the protein synthesis inhibition. DTx is taken into cell with vesicles and transferred to early endosomes. It was reported that cytochalasin D, Primaquine are blocked by the release of FA from early endosomes to cytosol and prevented the ADP-ribosylation of eEF2. Interleukin-1β (IL-1β) is a cytokine produced by macrophages and monocytes. IL-1β affects almost all cell types and its secretion is increased significantly in the pathogenesis of several diseases. In this present study, endogenous ADP-ribosylation activity of eEF2 was explored in IL-1β treated HUVECs with autoradiogram and western blot. FA release from early endosomes was examined with immunoflorescence microscopy. In conclusion, due to the IL-1β, endogenous ADP-ribosylation was increased and FA is held in early endosomes with DTx treatment.

L-794

Heterocomplex formation of the human AAA+ proteins VPS4A and VPS4B

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The AAA+ ATPase (**A**TPases **A**ssociated with Various Cellular **A**ctivities) VPS4 is essential for a myriad of cellular and pathological activities ranging from cell division, MVB biogenesis, retrovirus release, and cell membrane repair, but also regulation of the Ras signalling pathway. VPS4 assembles as an ATP-hydrolysing multimer and is assumed to solubilise a membrane-associated pool of the ESCRT-III family of proteins. Oligomeric state, ATPase activity, and interaction partners of non-mammalian VPS4 have been extensively studied. Lower eucaryotes possess only one isoform, whilst mammalian cells express two isoforms, VPS4A and VPS4B. It is controversely discussed whether they function separately or as a heterocomplex. We addressed this by directly measuring the interaction between the two proteins utilising analytical size exclusion chromatography. Further, we investigated the impact of oligomerisation on the ATPase activity either of the individual VPS4 isoforms or the heterocomplex. ATPase activity was measured under steady-state conditions using a NADH-coupled regeneration system. We observed that ATP induces heterocomplex formation. For VPS4A, but not VPS4B, we found a concentration-dependent homocomplex formation in the concentration range accessible. Both, hetero- and homocomplex formation lead to a stimulated ATP hydrolysis.

Abstracts

– 1. Protein Structure and Function –

L-795

Uni-molecular investigation of metals – D,L-histidines interactions with a protein nanopore

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Herein, we investigated the influence of chiral environment upon Cu^{2+} binding on an amyloid-beta (Aβ) substrate. Knowing that: (i) the spatial and physical properties of the Cu^{2+}-Aβ complex are distinct from that of the metal-free Aβ, and (ii) modifications involving Aβ's His-6; His-13; His-14 motif lead to changes in the Cu^{2+} coordination sphere, and thereupon Cu^{2+}-Aβ's physical and topological features, we studied how the replacement of L-His with their D-enantiomer alters Cu^{2+} binding to the mutated Aβ. The experimental approach was based upon stochastic sensing with an α-hemolysin (α-HL) pore, to monitor the interactions between Cu^{2+} and mutants of the $A\beta_{1-16}$ human fragment containing L- or D-His enantiomers in positions 6 and 13. The statistical analysis of single-molecule binding events reflecting peptide-α-HL reversible interactions in the presence of Cu^{2+}, led to the evaluation of dissociation constants of the metal toward the mutated Aβ substrate. We show that the Cu^{2+}-binding affinity decreases as L-His is replaced with D-His[1]. [1]Schiopu I.; Iftemi S.; Luchian T., *Langmuir* 2015, 31(1), 387-396.

We acknowledge PN-II-PT-PCCA-2011-3.1-0402, and GRL Grant (NRF- 2014K1A1A2064460).

L-796

Structural and functional analysis of ribosome:translocon complex at the lipid membrane interface

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In spite of the significant progress in understanding principles of membrane protein folding in cells, only limited structural information is available on the dedicated Sec and YidC-related machineries in physiologically relevant membrane environment. Here, we set out to combine biophysical analysis and structural biology aiming to elucidate the architecture of insertase:ribosome complexes upon membrane protein integration. Recently, we have employed fluorescence analysis to characterize YidC:ribosome and SecYEG:ribosome complexes formed at the membrane interface. Now, we extend the approach to study membrane protein insertion in native-like lipid environment of nanodiscs and newly designed nano-proteoliposomes. We observed that negatively-charged lipids, an essential component of biological membranes, prevent spontaneous insertion of hydrophobic polypeptides into the membrane. Fluorescence-based screening further allowed fine-tuning the lipid composition of those membranes to ensure functionality of SecYEG/YidC insertases and nano-molar affinity for insertase:ribosome complexes. The biophysical analysis has been used for designing on-going structural studies of these complexes by single-particle cryo-electron microscopy.

L-797

Spectroscopic and thermodynamic studies on the interaction of choline oxidase with ionic liquid

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Ionic liquids (ILs) are excellent solvents for enzyme-catalyzed reactions. On the other hand, investigation of the oxidoreductases behavior in the presence of ILs is crucial due to their industrial application. In this work, the interaction of an IL, 1-allyl-3-methylimidazolium bromide (AMIMBr), with Choline Oxidase (ChOx) was investigated by fluorescence spectroscopy. The results showed that the fluorescence quenching mechanism of ChOx in the presence of AMIMBr was static. The association constant (K_a) between IL and ChOx were calculated based on modified Stern–Volmer equation. The thermodynamic parameters, extracted from the fluorescence data, indicated that the interaction of IL and ChOx mainly involved van der Waals, hydrogen bonding, and stacking forces. Moreover, the change in the structure of ChOx molecules upon binding with IL was confirmed with synchronous fluorescence spectroscopy.

L-798

Fluorescence spectroscopy reveals conformational dynamics of the multifunctional protein GABARAP

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The gamma-aminobutyrate type A receptor-associated protein (GABARAP) from *H. sapiens* belongs to the MAP1 LC3 family of ubiquitin-like proteins, involved in vesicle transport and fusion events, in autophagy, and apoptosis (*FEBS J* **2009**, *276*, 4989). Structure determination of GABARAP by NMR and X-ray crystallography suggested degree of conformational heterogeneity undergoing on the micro- to millisecond timescale (*J Biomol NMR* **2002**, *22*, 97; *Biochem Biophys Res Commun* **2010**, *395*, 426). We apply fluorescence spectroscopy toolkit to investigate conformational dynamics in broad time range. The FRET distance landscape is investigated by analysis of fluorescence decay; FRET dynamics by correlation techniques (filtered FCS). We show how MFD toolkit (*Method Enzymol* **2010**, *475*, 455) can be applied to a dynamic system, providing complete fluorescence spectroscopic experiment from a single measurement. We find unexpected large-amplitude conformational dynamics of the N-terminal domain at tens of microsecond time-scale and a new thermally-excited state. This process may have implications for the regulation of GABARAP interactions and its function as a hub protein.

Abstracts

– 1. Protein Structure and Function –

L-799

Crowding by specific size of dextran switches the substrate specificity of acetylcholinesterase enzyme

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The cell cytoplasm, the red blood cell interior or the mitochondrial matrix are examples of crowded environments. The consequences of crowding by proteins and nucleic acids on physiological functions like enzyme catalysis, is poorly understood. Our interest here was to mimic such macromolecular crowding *in vitro* to understand how crowding by inert high molecular weight dextrans affect the catalytic activity of acetylcholinesterase (AChE) enzyme, specifically with different substrates. Hence, we monitored the catalytic rate of AChE in a medium crowded with dextrans of different sizes (15 to 2000 kDa) at different concentrations (0—20% w/w) employing two different substrates. Investigations revealed enhanced activity (> 2 fold increase) with 2-naphthyl acetate as substrate in comparison to 3-indoxyl acetate (IA), for all dextran sizes at low concentrations (0—10% w/w). Interestingly while AChE activity with 2-naphthyl acetate was specifically enhanced in presence of 200 kDa dextran, its activity with 3-indoxyl acetate in presence of same 200 kDa dextran, was drastically diminished in comparison to all other dextran sizes employed. Our results suggest that when dextran size matches enzyme, alteration of enzyme function is maximal, leading even to switch in substrate specificity.

L-800

An NMR investigation of activation of a sensor histidine kinase involved in vancomycin resistance

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Gram-positive bacteria are enclosed by a peptidoglycan layer, which protects the cell from environmental stresses. The antibiotic vancomycin binds to the d-Alanyl-d-Alanine-terminating peptide of peptidoglycan precursors, preventing their incorporation into the cell wall and resulting in the deterioration of this protective layer. A resistance mechanism has since evolved whereby modified d-Alanyl-d-Lactate-terminating peptidoglycan precursors, to which vancomycin cannot bind, are synthesised. This mechanism is activated by the sensor histidine kinase VanS, which phosphorylates the regulatory protein VanR in the presence of vancomycin. VanR upregulates the expression of genes that synthesise the modified precursor. VanS activity may be induced in the presence of various antibiotics aside from vancomycin, including the structurally similar teicoplanin and the unrelated moenomycin; thus the exact ligand of VanS has yet to be elucidated. Through the production of isotopically-labelled, full-length and truncated proteins, this study uses solution-state NMR to investigate the ligand-binding characteristics of two VanS proteins with distinct antibiotic sensitivity profiles, with the aim of identifying the ligands and exact binding sites of each.

L-801

How to build a host-pathogen interactome: a proof of concept for Schistosoma mansoni and its human host

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Vector-borne parasites represent a huge burden for human health worldwide. They evolved to evade the host immune response. Hence knowing which are the molecular players is important for both diagnostics and therapeutics.
We have started a high throughput screening of the interactions between secreted proteins of the human extracellular parasite *Schistosoma mansoni* and 78 selected components of the host extracellular matrix. *S. mansoni* lives attached to the veins of the portal system. Before attaining this final site, the infectious cercaria travels from the epidermis to the derma, then heads to the lungs, where it matures into the juvenile form and then travels to the final siege and matures in the adult stage.
Previous studies of the secretomes have evidenced a few common candidates. We have selected three of them: enolase, PDI and peroxiredoxin. Then we have screened with SPRi for their human partners. The positive hits were tested by SAXS, in order to reveal both the stoichiometry and the structure of the complexes. Here we present the results on SmEnolase, whose major interacting protein partners are plasminogen and tropoelastin, while the major interacting GAGs are dermatan and chondroitin sulfate.

L-802

Functional studie of a *Staphyloccocus aureus* flavohemoglobin

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Flavohemoglobins (FlavoHb) play a key role in bacterial resistance to nitrosative stress and NO signaling modulation. In this work, we cloned, expressed and characterized the flavoHb from the opportunistic pathogen, *Staphylococcus aureus*. We build a model structure by homology modeling showing structural similarities with those of other known flavoHbs. Interestingly the high sequence homology with the FlavoHb from *S. cerevisae* did not correlate with the enzymatic and kinetic properties which are much similar to those of *Escherichia coli*. *In vitro*, the enzyme accepts cytochrome *c* and oxygen as substrate. Based on this feature, we showed that depending on the proteins, the preferences for the electron acceptor entities are different and this is modulated by the chemical nature of the heme ligand. To make progress in understanding the catalytic mechanism of flavoHbs we investigated the enzyme properties, the effect of azole inhhibitors and the structure-function relationship in comparison to the well-known flavoHbs from the non-pathogenic bacteria. The mutation of key residues situated in the flexible region of the protein appeared to modify drastically the enzymatic properties suggesting that specific residue might play a crucial role in the regulation of the enzyme activity.

Abstracts

– 1. Protein Structure and Function –

L-803

The tethering to docking transition of extended tethers

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In vesicle trafficking, a selective tethering step is mediated by extended coiled-coil proteins at many cellular membranes. These membrane-associated tethering proteins reach out into the cytosol from their resident membrane, and their predicted length is far greater than the fusion machinery. How do the two membranes tethered in apposition at such a distance approach one another for fusion? Here, we reconstitute an endosomal minimal tethering machinery consisting of the small GTPase Rab5, phosphatidylinositol 3-phosphate, and EEA1. We define the determinants of tethering and investigate the process in a dual-beam optical trap. The measured tethering distance and physical properties of the molecule in the optical trap suggest a structural change in EEA1, observed directly by electron microscopy. In the light of well-defined physical theory for semi-flexible polymers, we describe a model in which changes in the mechanical properties of the tether can generate an effective force, resulting in a reduction of distance between the membranes. The proposed mechanism explains how extended coiled-coil proteins in trafficking could potentially bridge the distance between vesicle capture and docking.

L-804

Molecular structure prediction of NorA multidrug transporter from Staphylococcus aureus

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S. aureus uses NorA efflux pump to transport a broad range of antibiotics, especially fluoroquinolones, outside the cell, which induces multiple drug resistance. The biggest challenge in discovering new efflux pump inhibitors (EPI) that target *S. aureus* NorA, with structural-based methods, is to obtain a reliable structure of the transporter since it was not experimentally resolved.
NorA belongs to major facilitator superfamily (MFS) of membrane transporters. Sequence homology of MFS transporters is <30%, which excludes homology modeling as a viable option for obtaining a reasonable NorA structure. We used the I-TASSER program to predict the structure of NorA using three MFS transporters as structural templates. NorA structure has equilibrated within 30 ns of MD simulation, that lead to a marked improvement of Phi and Psi dihedral angles distribution.
Docking known NorA inhibitors (quinoline derivatives) in the equilibrated structure pointed to a hydrophobic pocket situated between helices 1-4 of NorA. Initial docking sores correlated weekly (r^2 of 0.47) to experimental activity (pIC_{50}), but rescoring with NNscore v2 program resulted in a better correlation (r^2 of 0.75) - which indicate that predicted NorA structure can be used in structure-based methods to identify novel EPIs.

L-805

Nanodiscs as a platform for molecular spectroscopy and X-FEL structural studies of membrane proteins

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Membrane proteins accomplish vital cellular tasks like responding to environmental stimuli as well as exchanging ions between the cytoplasm and the extracellular space. Therefore, the protein needs to rearrange and undergo concerted conformational changes.
We explore the potential of soluble phospholipid nanodiscs to resolve dynamic structures of single membrane proteins using ultra-short X-ray pulses from X-ray free electron lasers (X-FELs). This will represent a significant advance in crystallization-independent studies of membrane proteins. We developed a strategy to attach a gold nanoparticle as a diffraction tag via thiol interactions to an engineered cysteine residue on the membrane scaffolding protein encircling the nanodisc, which enables the automated collection of single particle diffraction patterns.
The reconstitution of membrane proteins into nanodiscs also allows spectroscopic studies of membrane proteins that yield information going beyond crystals. We reconstituted the conserved P-type ATPase CopA from *Legionella pneumophila* into nanodiscs, which is a key player in copper homeostasis. This enables spectroscopic studies of the allosteric couplings that are associated with ATP-powered copper transport in this enzyme in fully controllable lipid environments.

L-806

Two amino acids form a novel cytosolic gate in viral potassium channels

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Miniature viral potassium channels (Kcv) are a perfect model system to study fundamental structure-function relations: They are very similar to the pore of more complex potassium channels, in structure as well as in function; they are truly minimal with 78-120 aa per monomer and so far more than 80 orthologs have been identified.
One of the questions we focus on with this system is: 'What controls open probability and gating?' The physiologically most important gate in potassium channels is the cytosolic ('inner') gate. A crucial role in the formation of this gate has been attributed to a highly conserved glycine in the center of the pore-forming transmembrane helix. However, this so-called 'gating hinge' has been found missing or functionally not important in some channels, showing that there must be other mechanisms.
Kcv channels lack this canonical gating hinge. We analyzed chimeras and mutants of two highly homologous Kcv channels with different open probability in a cell-free expression setup. This strategy uncovered a two-amino acid motif near the cytosolic end of TM2, which controls the open probability in Kcv_{ATCV-1}-like channels, with clear rules, which amino acid "flavours" are needed to close the gate.

Abstracts

– 1. Protein Structure and Function –

L-807

Fully blind peptide-protein docking with pepATTRACT

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Peptide-protein interactions are ubiquitous in the cell and form an important part of the interactome. Computational docking methods can complement experimental characterization of these complexes, but current protocols are not applicable on the proteome scale. Here, we present the first fully blind flexible peptide-protein docking protocol pepATTRACT. It combines a rapid coarse-grained global peptide docking search of the entire protein surface with a two-stage atomistic flexible refinement. Global unbound-unbound docking yielded near-native models for 70 % of the docking cases when testing the protocol on the largest benchmark of peptide-protein complexes available to date. This performance is similar to that of state-of-the-art local docking protocols which rely on information about the binding site. Upon restricting the search to the peptide binding region, the resulting pepATTRACT-local approach outperformed existing methods. Docking scripts for pepATTRACT and pepATTRACT-local can be generated via a web interface at www.attract.ph.tum.de/peptide.html.

L-808

pH-controlled self-assembly of Influenza A virus scaffold protein M1

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Influenza virus M1 protein is crucial for both the RNP release and budding of new viruses. These processes take place at different pH, so the function of M1 is also pH-dependent. We combined SAXS with AFM in the whole physiologically relevant range of pH, from 4 to 7, and revealed the tendency of M1 to form helical structures, even in acidic medium, with the threshold of transition from predominantly monomeric form to complete helical structures at pH $\sim$ 6. AFM demonstrated fundamental difference in the M1 adsorption in neutral compared to acidic conditions. Performing the measurements at different ionic strengths, we estimated the charge of M1 in the concerned range of pH. Our results show that at pH of late endosome M1 significantly changes its charge meaning that electrostatics could be the main driving force in disassembly of the viral protein envelope. In addition, we demonstrated that assembly of M1 in helices should occur in a pH-independent manner. Modelling these processes using DLVO theory we estimated the energy of M1-M1 interaction. Work was supported by RSF (#15-04-00060), RFBR (#15-54-74002) and BMBF BIOSCAT (#05K20912).

L-809

Biophysical characterisation of FANCD2 CUE domain and LEU 231 ARG mutant

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Fanconi Anemia Complementation group D2 (FANCD2) protein comprises different functional domains including "Coupling of ubiquitin conjugation to endoplasmic reticulum" (CUE) domain. Earlier studies have shown that CUE domain deficient cells fail to repair the DNA intercrosslink. Therefore, to understand the mechanism of disease predisposition at structural level, we have purified FANCD2 (1-254) *wild-type* and cancer predisposing mutant at Leucine 231 Arginine protein. Purified proteins were subjected to Circular Dichroism & Fluorescence spectroscopy which reveals correctly folded secondary & tertiary structures. However, drastic difference was observed in Leu231Arg mutant at tertiary structure level. Thermal denaturation study concludes that *wild -type* and mutant protein unfold via intermediate formation. Dynamic Light Scattering study has predicted that FANCD2 (1-254) *wild -type* and Leucine 231 Arginine mutant protein exist in monomeric as well as in multimeric states. Comparative study of *wild -type* and mutant will further help in understanding the mechanism of folding and stability of FANCD2 CUE domain as well as structural implication of mutation.

L-810

Study of rabies virus by Differential Scanning Calorimetry

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Differential Scanning Calorimetry (DSC) has been used in the past to study the thermal unfolding of many different viruses. Here we present the first DSC analysis of rabies virus. We show that active, purified rabies virus unfolds cooperatively in two events centered at approximately 62 and 72 °C. Beta-propiolactone inactivated virus samples showed similar unfolding thermograms indicating that viral inactivation did not alter protein structure significantly. The initial unfolding event was absent in bromelain treated samples, causing an elimination of the G-protein ectodomain, suggesting that this event corresponds to G-protein unfolding. This hypothesis was confirmed by the observation that this initial event was shifted to higher temperatures in the presence of three monoclonal, G-protein specific antibodies. We show that dithiothreitol treatment of the virus abolishes the initial unfolding event, indicating that the reduction of G-protein disulfide bonds causes dramatic alterations to protein structure. The sharpness of unfolding transitions and the low error of the Tm values as derived from multiple analysis offers the possibility of using this analytical tool for efficient monitoring of the vaccine production process and lot to lot consistency.

Abstracts

– 1. Protein Structure and Function –

L-811

Towards antibiotic resistance strategies: the contribution of outer membrane permeability via porins

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Outer membrane protein porins are present in the bacterial cell membrane and their main function is to uptake nutrients from environment. Recent studies show these porins are selective to different substrates such as antibiotic molecules. Porins were reconstituted into planar artificial membrane and single channel current were recorded by using Port-a-patch and Orbit16 from Nanion. In this study, I characterized the interaction rates, at the single molecule level, by comparing the selectivity of different substrates among diverse porin homologues. I found that, the associative rate constant is determined not only by different amino acids present in the constriction zone, but also by the interaction between antibiotics and the residues in the constriction zone. Divalent ions such as magnesium can be used to change the electrostatic properties at specific points. So the effect of magnesium molecules has been checked in terms of the interaction kinetics. The observation of different binding manners shows divalent ions can be fast probe in protein-molecule interactions, i.e. the presence of magnesium change the interaction of zwitterionic molecules. The uptake mechanism of antibiotic molecules via bacteria membrane protein can be further characterized with the aid of MD simulation.

Abstracts

– 3. Live Imaging and Optical Microscopy –

L-813

A highly sensitive FLIM based assay for the detection of reactive oxygen species

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Nanoparticles hold great promise in biomedical science. However, their greater surface area to volume ratio, the higher chemical reactivity and biological activity may cause elevated intracellular reactive oxygen species; consequently damage DNA and proteins. Thus, there is a need to develop new sensitive methods for intracellular ROS detection. Using the oxidative stress reagent CellROX® Green, we developed an assay based on fluorescence lifetime image microscopy (FLIM). In comparison to intensity based measurements using conventional microscopes our assay is highly sensitive and reproducible. CellROX® Green is a DNA dye, which upon oxidation via ROS, is highly fluorescent and binds to the DNA in the nucleus. In our experiments we induced oxidative stress by incubating cells with different concentration of H_2O_2. In the FLIM experiments the cells show enhanced fluorescence upon addition of H_2O_2 and CellROX®. However, we were also able to discriminate those dyes that were bound to DNA from those in the cytoplasm based on their different fluorescence lifetimes. Using the lifetime of the dye bound to the DNA we determined a H_2O_2 concentration dependent binding curve, which can be used as a calibration curve to quantify the level of oxidative stress.

L-814

Investigating cell mechanics by atomic force microscopy

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Cell biology has seen a surge in mechanobiology-related research directed towards understanding how cells exert and respond to forces. Atomic force microscopy (AFM) not only allows direct examination of the nanoscale structure of cell membrane surfaces, it also provides unique opportunities to measure the nanomechanical properties of live cells. We have used a novel AFM imaging mode, PeakForce QNM®, to map the modulus of live, individual mammalian cells. These two-dimensional spatial maps provide both high-resolution and quantitative measurements of cell mechanics that directly correlate to cell topography. PeakForce QNM has demonstrated improved results in terms of resolution, speed, ease-of-use, and quality of the information delivered. Additionally, the different frequencies accessible with both Force Volume and PeakForce QNM provide new opportunities for examination of viscoelastic properties. Extending our studies to prokaryotes, we successfully used PeakForce QNM to detect variations in the modulus of bacteria cells that occur during cell division. By integrating PeakForce QNM imaging with fluorescence microscopy we were also able to demonstrate a correlation between changes in modulus and bacterial cell viability.

L-815

Counting Molecules in RESOLFT Nanoscopy

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Counting molecules in nanoscopy is an important problem. Many biological applications benefit from knowing quantitatively the spatial distribution of the imaged structures. In RESOLFT microscopy, these structures are labeled with switchable fluourescent proteins; their on-off switching kinetics are controlled to spatially confine the emission of fluorescence.
For quantitative microscopy, the image formation process has to be accurately modeled. We model the image formation in the RESOLFT microscope by calculating the spatiotemporal probability distribution of the fluorophores being in the on state. The average number of measured photons is proportional to the number of molecules in the on state. For switching fluorophores and in contrast to Poisson noise, the variance depends on the brightness quadratically. This quadratic dependence allows us to calculate the average brightness from the fluctuations of the signal in the image.
We develop an image deconvolution algorithm which, based on our model for the image formation in the RESOLFT microscope, gives an estimate of the brightness and the fluorophore number distribution in the image. For known number distributions in a simulation, we show the dependence of the error on signal to noise ratio and the true molecular parameters.

L-816

Visualizing the attractor of differentiation during myogenesis using Raman spectral imaging

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We have visualized the transition of cell state during C2C12 myogenesis using Raman spectral imaging. Formation of skeletal muscle is a complicated process involving fusion of myoblasts, formation of tubular myotube and arrangement of sarcomere structure which is difficult to evaluate at a single cell level. Here, we have visualized the cell state transition during C2C12 differentiation using Raman spectral imaging of cell nucleus in combination with PCA. Cell population seemingly having homogeneous cell state before the differentiation showed heterogeneity 3 days after induction of differentiation. After formation of myotubes, cell state became homogeneous again falling into different state from undifferentiated state. The result clearly illustrate the disappearance and reappearance of attractor in a differentiation landscape, where cells stochastically fluctuate its state at the midpoint of differentiation. Similar result was obtained during ESC differentiation, indicating that disappearance of strong attractor at the onset of differentiation may be common phenomenon.

Abstracts

– 3. Live Imaging and Optical Microscopy –

L-817

Particle dynamics at the inner wall of model blood capillaries studied by FCS

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At the interface of blood and tissue, the endothelial glycocalyx and associated blood plasma components form a dynamic matrix called endothelial surface layer (ESL) that affects the transfer of drugs and metabolites from blood to tissue. Although there is considerable knowledge about the structure and composition of this layer, there is a gap of experimental data regarding the dynamics of single molecules within the ESL. Therefore, we propose a FCS scheme with multiple, spatially shifted observation volumes that allows for the precise and sensitive measurement of both, diffusion and uniform translation in magnitude and direction, with high spatial resolution. Here, we report on the methodical issues of foci adjustment and distance estimation by artificial flow, and present first experimental results from FCS measurements in model vessels.

L-818

Characterizing structural features and affinities of protein complexes in living cells by MFIS-FRET analysis

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Förster resonance energy transfer (FRET) due to its sensitivity of distance has been widely used to investigate the structure and interaction of biomolecules. Multiparameter fluorescence image spectroscopy (MFIS) provides particular advantages to FRET imaging because all the fluorescence parameters are monitored simultaneously with picosecond accuracy, which allows for a comprehensive analysis on biological systems. Traditionally, a reduction in average donor lifetime or an increase of average FRET efficiency was used as an indicator for molecular interaction. However, such changes observed in FRET-imaging can have two reasons: (1) the conformational change or (2) change in fraction of FRET-active species. To resolve this ambiguity, we introduce a new sub-ensemble analysis method to directly visualize and quantitatively analyze both factors. Characterization of true FRET efficiency enabled us to detect even subtle FRET variations and provided crucial information about the structural properties of molecular complexes. Furthermore, from determined fraction of FRET-active species, utilizing the intrinsic cell-to-cell variations of protein concentration, we show that dissociation constant (K_D) of membrane-receptor interactions can be characterized *in vivo*.

L-819

Compressive cortical flow aligns actin filaments to initiate furrowing

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The actin cytoskeleton is a complex self-organized network that maintains cellular integrity while constantly undergoing rapid turnover. In this study we investigate how myosin induced cortical flows impact actin network organization and filaments alignment and thereby modify cell shape and initiate stable ingression. We perform our analysis in the early *C. elegans* zygote where cortical flows drive polarization as well as the formation of a pseudocleavage furrow, and at a later state during cytokinesis of the one-cell embryo. By imaging and analyzing actin architecture in this system in relation to myosin induced cortical flow, we observe that actin filaments align in converging and compressive flow. A quantitative analysis of the dynamics of flows, filaments orientation and cell shape changes, together with a theoretical description of alignment and order in the framework of a thin film of active and nematic fluid allows for a precise characterization of how deformation and shear in cortical flow gives rise to ordering of actin filaments, and how ordered actin filaments generate the forces that are required for forming an ingression. We thus identify the key physical principles that lead to the generation of an ingression for cytokinesis.

L-820

Superresolved molecular organization in fibronectin fibrils

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The extracellular matrix is vital for the mechanical integrity of connective tissues and for tissue homeostasis. During development and wound healing, cells assemble fibrils from the multimodular protein fibronectin. Although it is known which parts of the fibronectin molecule are necessary for fibril formation, intermolecular interactions and the hierarchical fibril structure have remained elusive.
Here we investigate native fibronectin fibrils in fixed cell culture using superresolution microscopy. Taking advantage of the complementary strengths of bioconjugate chemistry and immunohistochemistry for attaching fluorophores to specific sites on fibronectin, as well as of stepwise photobleaching and direct STORM for their localization, we determined the extension of single molecules, the periodicity, and the relative offset between adjacent molecules. These complementary data yielded a comprehensive picture of the molecular arrangement in fibrils.
In general, the presented methodologies offer diverse options to investigate also the structure of other protein fibrils in their native environment.
[1] SM Früh,..., I Schoen. ChemBioChem 15:1481 (2014)
[2] SM Früh*, I Schoen*,..., Nat. Commun. 6: 7275 (2015)

Abstracts

– 3. Live Imaging and Optical Microscopy –

L-821

Single molecule fluorescence microscopy of a large molecule penetrating into the human skin

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Here, we report on the application of laser-based single molecule total internal reflection fluorescence microscopy (TIRFM) to study the penetration of molecules through the stratum corneum (SC). The SC is the outermost skin layer, composed of keratin rich, metabolically inactive corneocytes, embedded in a highly ordered lipid matrix. This structure acts as an efficient penetration barrier, and thus presents a challenge in topical medication.
The model compound used is an amphiphilic molecule (ATTO-Oxa12) with a molecular weight >700 Da, larger than the often referenced 500 Da penetration cut-off. ATTO-Oxa12 was applied to excised human skin and the penetration through the SC into the viable epidermis was revealed by TIRFM of skin cryosections.
Single particle tracking of ATTO-Oxa12 within SC sheets obtained by tape stripping allowed us to gain information on the localization as well as the lateral diffusion dynamics of these molecules and thus of penetration pathways through the skin.

L-822

4 Colour STED nanoscopy with a single STED beam

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Mechanisms and dynamic processes in living cells result from the interplay of different proteins and molecules. A detailed understanding of such processes requires the observation of several molecular species at high spatial resolution at the same time.
Therefore the employed microscopy method needs to be able to discriminate several markers via different detection channels on the nanometer scale. For this purpose STED-microscopy (STimulated Emission Depletion) is an excellent tool. The doughnut-shaped STED-beam keeps molecules in the periphery of a Gaussian excitation beam in a dark state. The resulting focal spot is no longer subjected to Abbe's diffraction limit [1].
We present a set-up where up to 8 excitation wavelengths can be arbitrarily chosen. The detection spans a range of 150nm in 4 channels. Results show that 4 markers can be distinguished by their emission spectra with one excitation and one STED-beam. Thereby a resolution on the nm scale was reached for all structures. Analysis is done via a Non Negative Matrix Factorization algorithm [2].
References:
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Abstracts

– 4. Photobiophysics, Biological Electron and Proton Transfer –

L-823

Slow proton transfer accompanying the light-induced structural changes in bacterial reaction centres

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Light-induced conformational changes near the bacteriochlorophylls have been shown earlier to significantly increase the lifetime of the charge-separated state and lower the oxidation potential of the bacteriochlorophyll dimer. Changes in these parameters were linked to an increase of the local dielectric constant and a slow proton conducting pathway that connects the dimer and the periplasmic surface. In this work we systematically altered the dielectric properties of the surrounding by reconstituting the bacterial reaction centres (BRCs) into liposomes assembled from various lipids, where the head group charges and the fatty acid chain lengths were varied. The change of the potential of the dimer in the dark-adapted conformations was found to be nearly symmetrical in the ±20 mV range in liposomes with negative and positive head group charges. Contrarily, in the light-induced conformations, the largest decrease of the dimer potential (∼80 mV) was observed in the liposomes with zero net head group charge. The pH dependence of the large proton release was markedly altered by the combined influence of the head group charge and the packing of the liposomes and was found to be completely missing in RCs incorporated into liposomes with positively charged lipids.

L-824

Albumin-$[Ru(Phen)_3]^{2+}$ complex formation: the influence of albumin on oxygenation measurement *in vivo*

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A water soluble dichlorotris(1,10-phenanthroline)-ruthenium(II)hydrate ($[Ru(Phen)_3]^{2+}$) is a sensor of molecular oxygen presenting a minimal phototoxicity. In this work, the interaction of $[Ru(Phen)_3]^{2+}$ with human serum albumin (HSA) was investigated by time-resolved fluorescence spectroscopy and MicroScale Thermophoresis (MST). The Stern-Volmer constants were determined: $K_{sv} \sim 5x10^4\ M^{-1}$, $k_q \sim 7x10^{12}\ M^{-1}s^{-1}$. The high value of k_q refers to the formation of HSA-$[Ru(Phen)_3]^{2+}$ complexes and to a static quenching of tryptophan. MST experiments revealed that Kd for the complex formation is 64 μM. The influence of HSA on the $[Ru(Phen)_3]^{2+}$ phosphorescence lifetime was measured in single cells with a PLIM system. The $[Ru(Phen)_3]^{2+}$ lifetimes were determined in different cellular compartments and ranged between 0.8 and 2 μs. Moreover, reliable measurements of the oxygen level using $[Ru(Phen)_3]^{2+}$ as a probe was performed in the blood stream and in the extravascular space of the CAM. This work was supported by 7FP EU Project CELIM 316310.

L-825

Nitric oxide is produced through mitochondrial reverse electron transfer

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Heart phosphorylating electron transfer particles (ETPH) showed a NAD^+ reductase activity of 63.9±3.3 nmol/min.mg protein, sustained by reverse electron transfer at expenses of ATP and succinate. This activity was inhibited by rotenone (99%), oligomycin (98%) and m-CCCP (93%). ETPH produced NO at 1.1 ± 0.1 nmol/min.mg protein. In the presence of the compounds needed to carry out the reverse electron flow, ETPH produced 0.96 ± 0.07 nmol NO/min.mg protein, suggesting that NO production can be supported by electrons derived from the respiratory chain. Rotenone completely inhibited mtNOS activity supported by reverse electron transfer measured in ETPH, but that inhibitor did not reduce the activity of isolated nNOS, indicating that the inhibitory effect of rotenone on NO production by ETPH is due to an electron flow blockage and not to a direct action on NOS structure. A mitochondrial fraction enriched in complex I produced 1.72 ± 0.18 nmol NO/min.mg protein and reacted with anti-nNOS antibodies, suggesting that complex I physically and functionally interacts with the NOS enzyme. These data show that mitochondrial NO production is supported by reverse electron flow of the respiratory chain, this latter a phenomenon that could occur in physiopathological conditions.

L-826

Singlet oxygen distribution in lipid membranes governs photodynamic oxidation of the target molecule

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The efficacy of photosensitizers (PS) used in photodynamic therapy for cancer by using planar lipid bilayers have been studied. Membrane adsorption of the molecules and their destruction were monitored via changes of the boundary potential at the water/membrane interface. Aluminum(III) phthalocyanines with different numbers of sulfonate groups and various water soluble free base or phosphorus(V) porphyrins derivatives served as PS and the styryl dye, di-4-ANEPPS, as singlet oxygen (^1O) target. We used fluorescent correlation spectroscopy to obtain absolute surface densities. Upon illumination, PS produced ^1O, which in turn oxidized the target. As a result, the boundary potential dropped at the rate at which target oxidation occurred. This rate decreased with the target's membrane surface density, indicating that the target acts as ^1O quencher. It increased with PS density until a PS level sufficient for ^1O quenching was reached. The efficacy of ^1O quenching by the different target molecules and by PS varied according to their immersion depth into the hydrophobic core of the lipid bilayer, indicating that ^1O steady-state concentration is highest in the hydrophobic core and decreases towards the membrane surface.
Supported by Russian Scientific Fund [1] 14-13-01373

Abstracts

– 4. Photobiophysics, Biological Electron and Proton Transfer –

L-827

The direct measurements of charge transport in *Shewanella Oneidensis* MR-1 conductive appendages

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The direct transport measurements in biological nanowires (pili) is rather complex problem that excites the minds of scientists within last two decades. First of all, the investigation of charge-transport mechanism requires the nanowire to be kept in the most intact state both in chemical and physical sence, while in literature pili are used to be chemically fixed. The next problem arises with the use of conductive-probe atomic force microscope in the point-spectroscopy mode due to shortcomings at nanometer scales. Finally, the use of focused ion beam for contacts fabrication requires regime adaptation due to various restrictions of ion beam induced deposition of metals. In current work we present methodological solution for: (1) spatial organization of contacts to the object of interest; (2) scanning tunneling spectroscopy; (3) DC measurements and (4) impedance spectroscopy of single nanowire of Shewanella oneidensis MR-1. Most intriguing results were acquired using impedance spectroscopy. The analysis of Nyquist plot of hodograph has shown its sensitivity to relative humidity, ligth irradiation and contacts' properties. These data contradict the results reported earlier by other groups.

L-828

A decaheme cytochrome as a molecular electron conduit in dye-sensitized photoanodes

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In nature, charge recombination in light-harvesting reaction centers is minimized by efficient charge separation. Here, it is aimed to mimic this by coupling dye-sensitized TiO_2 nanocrystals to a decaheme protein, MtrC from *Shewanella oneidensis* MR-1, where the 10 hemes of MtrC form a ≈7-nm-long molecular wire between the TiO_2 and the underlying electrode. The system is assembled by forming a densely packed MtrC film on an ultra-flat gold electrode, followed by the adsorption of approximately 7 nm TiO_2 nanocrystals that are modified with a phosphonated bipyridine Ru(II) dye (RuP). The step-by-step construction of the MtrC/TiO_2 system is monitored with (photo) electrochemistry, quartz-crystal microbalance with dissipation (QCM-D), and atomic force microscopy (AFM). Photocurrents are dependent on the redox state of the MtrC, confirming that electrons are transferred from the TiO_2 nanocrystals to the surface via the MtrC conduit. In other words, in these TiO_2/MtrC hybrid photodiodes, MtrC traps the conduction-band electrons from TiO_2 before transferring them to the electrode, creating a photobioelectrochemical system in which a redox protein is used to mimic the efficient charge separation found in biological photosystems.

L-829

Nitric oxide interacts with complex III producing antimycin-like effects

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The interaction of NO with mitochondrial complex III was studied using submitochondrial particles (SMP) from bovine heart and GSNO and SPER-NO as NO sources. Complex II-III activity (222±4 nmol/min.mg protein) was inhibited by ∼50% in the presence of 500 μM GSNO or 30 μM SPER-NO, in both cases ∼1.25 μM NO. This effect was not due to the inhibition of complex II activity. Complex II-III activity was also decreased (36%) by endogenously produced NO. GSNO (500 μM) reduced cytochrome b_{562} by 71%, in an [O_2] independent manner, and produced a hyperbolic increase in $O_2^{\bullet -}$ (up to 1.3±0.1 nmol/min.mg protein) and H_2O_2 (up to 0.64±0.05 nmol/min.mg protein) productions. Succinate-energized SMP showed an EPR signal (g∼1.99) compatible with a stable semiquinone, which was increased by GSNO and SPER-NO. These signals were not modified under N_2 atmosphere, discarding the effect of NOx species. The absence of an EPR signal compatible with a mono nitrosyl iron complex (MNIC) allows excluding the disruption of the Fe_2S_2 cluster by a MNIC formation. These results show that NO interacts with ubiquinone-cytochrome b area independently on [O_2] producing antimycin-like effects, i.e. inhibition of electron transfer with an [$UQH^{\bullet}$]ss enhancement which sustains an increase in $O_2^{\bullet -}$ and H_2O_2 production rates

L-830

Electron, proton and water transfer along the catalytic cycle of the Photosystem II

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Water oxidation in photosynthetic organisms occurs through the five intermediate steps S_0-S_4 of the Kok cycle in the Oxygen Evolving Complex of Photosystem II. Along the catalytic cycle, four electrons are subsequently removed from the Mn_4CaO_5 core by the nearby tyrosine Tyr-Z, which is in turn oxidized by the chlorophyll special pair P680. Recently, two Mn_4CaO_5 conformations, consistent with the S_2 state (namely S_2^A and S_2^B models) were suggested to exist, perhaps playing a different role within the S_2 to S_3 transition. Here, we report QM/MM simulations revealing that upon such oxidation the relative thermodynamic stability of the two previously proposed geometries is reversed, the S_2^B state becoming the leading conformation. In this state a proton coupled electron transfer is spontaneously observed on about 100 *fs* at room temperature. Upon oxidation, the Mn_4CaO_5 cluster releases a proton from the nearby W1 water molecule to the close Asp-61 on the *fs* timescale, thus undergoing a conformational transition increasing the available space for the subsequent coordination of an additional water molecule. The results can help to rationalize previous spectroscopic experiments and confirm that the water-splitting reaction has to proceed through the S_2^B conformation.

Abstracts

– 4. Photobiophysics, Biological Electron and Proton Transfer –

L-831

Fluorescence quenching of Badan and Prodan by tryptophan in cytochromes P450 and micelles

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Photoinduced electron transfer (PET) is a new quenching mechanism for (dimethyl)aminonaphthalene dyes that are often used as solvatochromic polarity probes or FRET donors and acceptors. Fluorescence of the two typical dyes Badan and Prodan is quenched by tryptophan in Brij® 58 micelles as well as in two cytochrome P450 proteins (CYP102, CYP119) with Badan covalently attached to a cysteine residue. Formation of nonemissive complexes between a dye molecule and tryptophan accounts for about 76% of the fluorescence intensity quenching in micelles, the rest is due to diffusive encounters. Cyclic voltammetry of Prodan in MeCN shows a reversible reduction peak at –1.85 V vs. NHE that becomes chemically irreversible and shifts positively upon addition of water. The excited-state reduction potential of Prodan (and Badan) is estimated to vary from about +0.6 V (vs NHE) in polar aprotic media (MeCN) to approximately +1.6 V in water. Tryptophan quenching of Badan/Prodan fluorescence in CYPs and Brij® 58 micelles involves tryptophan oxidation by excited Badan/Prodan, coupled with a fast reaction between the reduced dye and water.

L-832

Modelling proton transfer pathways in Cytochrome c oxidase

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Cytochrome c oxidase (CcO) catalyzes the reduction of molecular oxygen to water and utilizes the chemical energy to establish an electrochemical gradient by pumping protons across the membrane it is embedded in. The proton transfer occurs through two distinct channels (D- and K-) by the formation and cleavage of covalent bonds along a hydrogen bond network. By simplified proton transfer models, resembling the channel conditions, we analyzed the proton transfer using different quantum mechanical energy functions and the influence of several degrees of freedom (e.g rotation and translation of water molecules) on the proton transfer. The impact of water mobility and the optimal positioning of proton-donor and -acceptor pairs on the proton transfer probability was investigated by MD simulations of the model systems. Optimal proton transfer pathways are then determined from computing proton transfer networks explicitly sampling all relevant degrees of freedom. In addition to the model calculations we performed MD simulations with CcO embedded in a membrane. Both, the D- and K-channel show significant differences in the hydrogen bond networks and water mobilities, dependent on the protonation state.

Abstracts
– 5. Molecular Motors –

L-833

Replication dynamics of the human mitochondrial DNA polymerase

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Mitochondrial DNA polymerase gamma (Pol γ) is the sole polymerase responsible for replication of the mitochondrial genome (mtDNA). It is well established that defect in mtDNA replication lead to mitochondrial dysfunction and disease. To date, approximately 260 disease mutations in Pol γ have been identified, which places Pol γ as a major locus for mitochondrial disease. To understand the molecular basis of these diseases, it is important to define the molecular mechanisms that govern the enzymatic activity of Pol γ. To this end, we are using optical tweezers to study the real time kinetics of individual Pol γ molecules. We use different experimental geometries to investigate the primer extension and strand displacement activity of the polymerase, and the effect of the mitochondrial Single Strand Binding (SSB) proteins on these activities.

L-834

Measurements of singlefluorescent motor proteins: the right way

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Cytoskeletal motor proteins are required in many cellular processes, such as intracellular transport and mitosis. Therefore, the biophysical characterization of motor protein movement along their filamentous tracks is essential. Commonly, stepping motility assays are used to determine the stepping and detachment rates of various molecular motor proteins by measuring their speed, run length and interaction time. However, comparison of these results proved to be difficult because the experimental setup, the experimental conditions and data analysis can influence the results. Here, we describe a method to evaluate traces of fluorescent motor proteins and propose an algorithm to correct the measurements for photobleaching and the limited length of the filaments. Additionally, bootstrapping is used to estimate statistical errors of the evaluation method, which was tested with numerical simulations as well as with experimental data from kinesin-1 stepping experiments. Results show that the stepping rate of kinesin-1 is highly dependent on the temperature and experiments with different microtubule preparations show that the run length of kinesin-1 is independent of the microtubule length distribution as well as the nucleotide state of the stabilized microtubules.

L-835

Role of interfacial friction for flow instabilities in a thin polar ordered active fluid layer

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We construct a generic coarse-grained dynamics of a thin inflexible planar layer of polar-ordered suspension of active particles, that is frictionally coupled to an embedding isotropic passive fluid medium with a friction coefficient. This provides a unified framework to describe the long wavelength behaviour of a variety of thin polar-ordered systems, ranging from wet to dry active matters and free standing active films. Linear instabilities around a chosen orientationally ordered uniform reference state depend sensitively on the frictional coefficient. Based on our results, we discuss estimation of bounds on friction coefficients in experimens.

Abstracts

– 6. Supramolecular Assemblies and Aggregation –

L-836

How do different protein interaction types influence mechanical properties and the assembly pathway of CCMV capsids - a multiscale simulation study

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We use a combined approach of classical atomistic and different coarse grained (CG) simulation levels to investigate the 180-protein icosahedral capsid of Cowpea Chlorotic Mottle Virus (CCMV).
First, the unstructured regions of the CCMV capsid proteins are studied by applying a suitable CG model together with clustering algorithms and free energy reweighting methods. The CG simulations combined with backmapping and subsequent atomistic simulations allow us to propose a multiconformational ensemble for the experimentally-unresolved regions of the pentameric protein interface.
In a second step, we use atomistic reference simulations to refine a CG protein model in such a way that it reproduces the elastic behavior of individual proteins and protein dimers in solution. The obtained model correctly predicts structural and elastic properties of bigger aggregates and mechanical properties of an entire virus capsid when compared to Atomic Force Microscopy experiment.
Detailed analysis of the simulated rupture process allow us to propose an assembly model through well-defined oligomeric intermediate states, where the assembly order is regulated by the strengths of the interfacial binding, with a subsequent post-assembly reinforcement of weak spots by cooperative folding.

L-837

Simulations of amphiphilic peptides reveal factors governing mineralization at interfaces

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Biomineralization is the intricate process employed by living organisms to form minerals to build skeletal structures and shells. Rapaport and coworkers (*J. Am. Chem. Soc.* 2000, 122, 12523) have designed an important class of self_assembling amphiphilic peptides that form hydrogels in bulk and, upon addition of ions, enhance bone tissue regeneration.
Human orthopedic conditions, such as osteoporosis, are a direct consequence of poorly orchestrated biomineralization. Deciphering the molecular mechanism of this vital yet poorly understood process is thus essential for the development of therapeutic approaches. Our study fills this void by revealing the factors that might promote formation of stable aggregates, and subsequent biomineralization events.
Here we identified the effects of various aspects of the peptide sequence on aggregate stability and ion_peptide interactions by molecular simulations. Our results reveal that peptides with proline as terminal residues formed more strongly ordered aggregates compared to those with phenylalanine. Aggregate stability was also found to be influenced by the nature of the side_chain groups of the peptides. Simulations in the presence of various ions showed how the ions influence aggregate stability in a side_chain_dependent manner.

L-838

Understanding fibrin protofibrils at the molecular level through a new coarse-grained approach

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The process of fibrin clot formation, together with the mechanical properties that arise from its topology are subjects of high bio-medical interest. Despite of recent experimental advances several limitations to understand such structures at the molecular level still remain.
Computer simulations using atomistic molecular dynamics are well established as a theoretical tool for structural characterisation to complement experimental studies, but its computational expense means that simulations of fibrin aggregation are unfeasible, even with modern supercomputers.
To overcome this limitation, we have developed a new coarse-grained model for proteins known as FFEA (Finite Element Molecular Analysis) which treats proteins as a non-rigid continuum subjected to thermal fluctuations. In this work, we adjusted the Young's modulus, which defines molecular flexibilty within FFEA, for the fibrin monomer using data from forced unfolding single-molecule experiments.
In addition, we are working on a bottom-up parameterisation method that will allow us to model specific sequence-dependent interactions, opening the door to study pathological mutations, as well as to the rational design of a new drugs that modulate the interaction strengths of fibrin.

Abstracts

– 7. Membrane Structure and Domains –

L-839

Amphipathic peptides induce formation of lipid pores

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We theoretically address formation of through pore by amphipathic peptides in lipid membrane. The membrane is considered as continuous liquid-crystal medium, subject to elastic deformations of splay, tilt, and lateral compression/stretching. Energetic landscape of peptides interaction at the membrane surface favors opposition of parallel peptides at a distance of several nanometers from each other. The membrane between the peptides becomes thinner by several angstroms. The final structure is the pore with the peptides lining its equator. The energy of the pore is lower than the energy of two opposing peptides if their diameter is more than 1 nm.
The work was supported by Russian Science Foundation project # 15-14-00060.

L-840

FCS techniques on monolayer systems for measuring protein dynamics and aggregation behaviour

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Sar1, a component of the coat protein complex II (COPII), and the amphiphysin II N-BAR dimer both have amphipathic α-helices, which insert only into the proximal leaflet of a phospholipid membrane. Phospholipid monolayers are therefore convenient model systems for analyzing these types of protein-lipid interactions.
A standard Langmuir film balance setup allows observing changes in surface pressure and surface area. However, the concentration of the bound protein and its diffusion properties are not readily accessible.
To overcome these limitations, we combined a Langmuir film balance with a confocal laser scanning microscope (CLSM) for the purpose of fluorescence correlation spectroscopy (FCS) and raster image correlation spectroscopy (RICS) measurements.
Since the monolayer has a thickness of only a few nanometers, it is important to accurately know the focal shape and width. This is achieved by using the known pixel size of images and a z-scan RICS analysis on a lipid monolayer as a thin sample. With this calibration we are able to determine absolute diffusion coefficients of proteins and lipids in a monolayer. We can also deduce the size distribution of protein clusters on the monolayer by calculating their influence on the temporal and spatial part of the correlation functions.

L-841

Membrane insertion of the small GTPase Arf1 causes tubule formation via the bilayer-couple mechanism

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The small GTPase Arf1 of the RAS superfamily plays an important role in vesicular trafficing. On the Golgi membrane, the formation and fission of coat protein I (COPI) transport vesicles proceeds via local deformation of the lipid bilayer by a curvature generating COPI protein coat. The assembly of this complex is initiated by the GTPase Arf1 in a nucleotide-dependent manner. After GDP/GTP exchange, soluble Arf1 becomes membrane bound by insertion of its myristoylated N-terminal amphipathic helix (myrAH) into the proximal leaflet of the Golgi membrane. The subsequent liberation of transport vesicles requires the full COPI complex and has been observed in vivo and in vitro. As the role of Arf1 in the process of curvature induction has not been fully elucidated, we have studied binding and incorporation of recombinant S. cerevisiae Arf1p into lipid mono- and bilayers using binding assays with a Langmuir film balance setup and artificial, unilamellar liposomes. We observe myristoylation-dependent binding to membranes and an increase in membrane surface area upon addition of Arf1p. Confocal laser scanning microscopy and cryo electron microscopy reveal highly curved membrane structures upon incorporation of myristoylated Arf1p. Our results support a mechanism of curvature induction based on the bilayer couple theory.

L-842

Nanolithographic lipid patterns on self-assembled monolayers

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Patterning of lipids is an interesting experimental strategy relevant both to biophysical research and to biotechnological applications. In this study, we have investigated dip-pen nanolithography (DPN) of lipids on self-assembled monolayers (SAMs), serving as substrates with well-defined surface properties. We employed three different types of surfaces: a hydrophobic eicosanethiolate SAM (C_{20}), a moderately hydrophilic triethylene glycol-terminated SAM (EG_3) and a heterogeneous SAM comprising hydrophobic alkyl chains dispersed in an ethylene glycol matrix (mixed EG). Lipid printing on the C_{20} SAM resulted in arrays of dots (diameter d=1μm and height h=200nm). On the EG_3 SAM, the lipid transfer rate was ten times higher and DPN resulted in disk-like features with d up to 80μm and h= 1 nm respectively. The mixed EG SAM was an intermediate case in terms of lipid spreading. The contrast in lipid spreading rate allowed us to fabricate micropatterned SAM substrates, in which the lipid features were efficiently confined by the C_{20} SAM lines and grids. In the future, such lipid microstructures could serve as models for lipid membrane transport, drug formulation and delivery systems.

Abstracts

– 7. Membrane Structure and Domains –

L-843

A novel membrane label for STED nanoscopy of living cardiomyocytes

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In cardiomyocytes the fast and cell-wide propagation of rhythmic action potentials crucially depends on the architecture and composition of the sarcolemma and its invaginations that form a transverse-axial tubular system (TATS). Both sarcolemma and TATS are enriched in cholesterol- and protein-rich membrane nanodomains that are strongly involved in cell signaling. Suitable live cell membrane labels to investigate these nanodomains are thus of high scientific interest.
Here we report on the first application of the novel fluorescent membrane label Chol-KK114 for STED nanoscopy of living mouse cardiomyocytes. Chol-KK114 enables fast and nontoxic in vivo labeling of cholesterol-rich cardiac membrane nanodomains.
We observed complex sarcolemmal and intracellular cholesterol nanodomains sized far below the confocal resolution limit. These signal patterns are rich in detail and highly cell type specific. On the sarcolemma, we identified individual cholesterol-rich membrane nanodomains with a mean diameter of 73 nm and arrangements into ring structures and patches. Rings have a mean diameter of 200 nm. Patches are of variable sizes and show distinct substructures.
Conclusively, we established a novel membrane label for superresolution microscopy of nanodomains in living primary cells.

L-844

Identifying mechanosensing and mechanotransducer elements within caveolae

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Mechanotransduction has emerged as an important field at the interface between physical cues and cellular signalling. Caveolae, small omega-shaped invaginations of the plasma membrane, have been proposed as key elements in sensing and transducing mechanical forces, although the specific contribution of the invagination versus the major protein components (caveolin-1/Cav1 and cavin-1/PTRF) is still unknown. Previous studies have shown that caveolae can flatten out in the plasma membrane in a process both ATP and actin independent; however, work in our lab showed that caveolae can also flatten in a process that requires actin polymerization, suggesting that a different response is obtained depending on the applied force. In order to distinguish between different types of forces we took advantage of the magnetic tweezers technique; coating magnetic beads with either Fibronectin or Concanavalin A we were able to study forces that affect integrin-cytoskeleton axis or forces that affect plasma membrane, respectively. To analyse the role of Cav1 and PTRF alone in this process, we developed genetically modified fibroblast lines that express caveolae components alone or the whole structure.

L-845

Does the tail wag the dog? - Cholesterol's aliphatic side chain modulates membrane properties

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The interactions between cholesterol and others membrane molecules determine important membrane properties. Even small changes in tetracyclic ring structure of cholesterol have crucial influence to these interactions.
We studied the influence of the cholesterol side chain on lipid ordering, lateral diffusion and membrane permeability using synthetic cholesterol derivatives with an *iso*-branched or an unbranched aliphatic side chain of different length.
Surprisingly, cholesterol's side chain is crucial for membrane properties and responsible for more than half of the phospholipid condensation in bilayers. For *iso*-branched side chains the investigated membrane properties are strongly dependent on the sterol side chain length. Shorter as well as longer side chains lead to smaller effects compared to cholesterol (*iso*-branched chain of 8 carbons). For unbranched sterol side chains these differences are smaller and a longer side chain is needed to achieve cholesterol like effects.
Therefore, also the sterol side chain of cholesterol plays a key role for membrane properties of cholesterol which has significant influences for the interaction with phospholipids.

L-846

Coarse-grained MD simulations of lipid anchoring to membrane domains

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Many proteins are bound to a membrane via post-translational modifications such as lipid anchors which are vital for their function. This work uses coarse-grained (CG) molecular dynamics (MD) simulations to investigate effects of the membrane anchor on the lipid bilayer and *vice versa*. The simulated lipid anchor, consisting of two geranyl-geranylated cysteine (CYGG) residues, originates from an all-atom (AA) model of the small GTPase Rab5. For computational efficiency, not the entire protein but only the N-terminal ten amino acids incl. the two CYGG residues were simulated. The simulations were performed with MARTINI, whereby the CG lipid anchor was parameterized against AA simulations. A variety of lipid bilayers, consisting of POPC (palmitoyl-oleoyl-phosphatidylcholine), cholesterol and PSM (n-palmitoyl-sphingomyelin) were modeled using a recent procedure described by Wassenaar et al. The established CG model of the CYGG anchor reproduced the AA properties in terms of both bond lengths and (torsion) angle distributions, as well as gyration radius and anchoring depth very well. According to our simulations, the CYGG anchor is not uniformly distributed but enriches in PSM domains. The structural properties are only minor affected by the type and composition of the lipid bilayer.

Abstracts

– 7. Membrane Structure and Domains –

L-847

Interaction of a major rubber protein (REF) with lipids extracted from *H. brasiliensis* latex

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The rubber particle of *Hevea brasiliensis* latex is a core of polymer surrounded by a membrane made of lipids and proteins, including a protein named Rubber Elongation Factor (REF). Whereas it is agreed that the particle core is made of poly-isoprene, the organization of its surface membrane is still controversial: double-layer, mixed monolayer and shell interfacial models were proposed by Cornish (1999), Nawamawat (2011) and Rochette (2013), respectively.
Recently, an approach in Langmuir films was applied by Berthelot *et al.* (2014) to study the interactions of recombinant REF with synthetic lipids. In this study, we used surface pressure kinetics, ellipsometry, Brewster angle microscopy and PM-IRRAS to investigate the interactions of recombinant REF and native lipids extracted from latex collected in Chanthaburi, Thailand. Monolayers of native phospholipids (PL) and glycolipids (GL) were successfully formed at the interface. REF adsorbs strongly into native PL and GL monolayers with higher disorganization of GL than PL.

L-848

Dynamic behavior of sphingomyelin in lipid rafts examined by fluorescence experiments and 2H NMR

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Lipid rafts are sphingomyelin (SM) and cholesterol (Chol)-rich microdomains, which are thought to have significant functional roles in cellular processes such as signal transduction. Despite such biological significance, the molecular basis of lipid raft formation is not fully understood. Thus, it is necessary to clarify the atomistic behavior (dynamics and interaction) of lipid molecules in lipid rafts.
In this study, we performed fluorescence experiments (anisotropy and lifetime) in stearoyl SM and glycerophosphocholine (PSPC) membranes in the presence of Chol. A comparison of the results with 2H NMR data, suggested that the membrane order obtained from the fluorescence experiments showed a similar temperature dependency as those of the 2H NMR data. More importantly, fluorescence lifetime experiments suggested the presence of two types of nanodomains with different ordering properties on a nanosecond time scale for both lipid membranes. In addition, we observed the difference of the relative abundance of each domain in SM and PSPC membranes.

Abstracts

– 8. Single Molecule Biophysics –

L-849

Structural insights into blinking of photoconvertible fluorescent proteins used as markers in PALM

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Single-molecule localization microscopy (e.g. PALM) is a powerful technique to investigate cellular nanostructures quantitatively and dynamically. However, the use of PALM for molecular counting or single-particle tracking remains currently limited by the marked tendency of photoconvertible fluorescent protein (PCFPs) to blink, ie repeatedly enter long-lived dark states. Bright PCFPs with reduced blinking are thus highly desirable. We rely on a combination of X-ray crystallography and single-molecule microscopy to unravel the mechanisms of blinking in PCFPs and rationally engineer low-blinking variants. Here, we designed mEos2-A69T and Dendra2-T69A to show that the blinking behaviors observed in mEos2 and Dendra2 variants are largely controlled by the side-chain orientation of Arg66, a highly-conserved residue in Anthozoan PCFPs. Our data suggest that Arg66 affects both the bleaching and the on-to-off transition quantum yields, resulting in widely different apparent blinking behaviors. Moreover, the lifetime of the off-state, which largely dictates the efficacy of blinking correction procedures, is also influenced by the Arg66 conformation. The present work will facilitate future engineering of bright and low-blinking PCFPs suitable for PALM microscopy.

L-850

Nanopore tweezers: voltage controlled trapping and releasing of analytes

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In recent years, a wide number of single-molecule protocols based on nanopore technology have been proposed in literature. The working principle of nanopore sensing is actually very simple. A nanopore connects two chambers containing an electrolyte solution. When a voltage is applied between the chambers a ionic current sets in. If a macromolecule translocates through the pore or it is blocked at the pore mouth, the ionic current is altered. From the current signal variation, information about the molecule (e.g. base sequence in the case of nucleic acids or folding state for proteins and peptides) can be inferred.
In this study, we rationalize and extend an approach, dubbed nanopore tweezer, originally proposed in Asandei, A, et al. (Scientific Reports, 5:10419, 2015), to control the residence time of an analyte inside a nanopore. The method can be applied to both biomolecules and nanoparticles. The only requirement is the strong polarity of the passing species, a property that can be obtained, for instance by adding a positive and negative tails at opposite ends of it.

L-851

Multi-conformation biomolecule structure determination by high-precision FRET and simulations

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A comprehensive methodology and a toolkit for FRET-enhanced modeling of biomolecules and their complexes are presented. The demonstrated approach enables to recover representative conformations for multiple observed states of the investigated biomolecular system. The toolkit is available from the authors including:
1. FRET-restrained docking and Metropolis Monte Carlo simulation tool for assembling structural units and determining the confidence levels of the generated models.
2. FRET-screening tool for assessing an arbitrary set of conformations (crystal structures, simulations etc.) with respect to their agreement with FRET measurements.
3. NMSim geometric simulations for extensive sampling of the conformational space.
4. FRET-enhanced MD simulations for refining conformations generated by coarse-grained sampling.
5. Experiment planning tool for determining efficient labeling positions and distance pairs for FRET measurements based on *a priori* knowledge on conformational changes.
An application on T4 Lysozyme with 24 FRET restraints, unreveling previously unknown state will be presented.

L-852

RNA junctions structure and distance determination via accurate single-molecule high-precision FRET

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We apply single-molecules multi-parameter fluorescence detection (MFD) and ensemble Time-Correlated Single Photon Counting to perform FRET studies on RNA three- and four-way junctions (4WJs, 3WJs) of high precision in distance better than 1% of the Förster radius.
We studied junction conformations influenced by sequences and bulges on one 4WJs and six 3WJs. Overall 275 FRET pairs were measured with single-molecule MFD and analyzed with the toolkit for FRET distance determination and further model generation. In 4WJ we detect three different conformers. In 3WJs we find only one predominant conformer. Junction geometry was described in terms of Euler angles between helices. The results suggest that the sequence dictates a junction specific conformation within this topology space. Furthermore we report that bulges in the junction region determine orientation and rotation of helices, inducing coaxial stacking between two of them.

Abstracts
– 8. Single Molecule Biophysics –

L-853

Changes in the levels of some trace elements in workers occupationally exposed to low levels of benzene

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Long-term occupational exposures to benzene have been reported to have deleterious effects on various body organs and systems**.** The present study was designed to evaluate the effects of chronic exposure to benzene on the changes in some trace elements in the plasma and RBCs of affected individuals. One hundred and sixty males occupationally exposed to low levels of benzene for more than two years formed the study group with age range between 20-50 years. Sixty healthy males with matched ages and similar living conditions and dietary habits, who were not directly exposed to benzene, served as control group. Of the 25 assayed elements in plasma and RBCs from venous blood of recruited individuals , statistically significant differences between the chronically exposed and the non-exposed workers were found in the levels of 17 elements, either in the RBCs, plasma or both, while such statistical differences were not detected for the other 8 elements The results showed that in RBCs major increases were detected in the levels of Al, Cd, Co, Pb, Hg, Ni, P and Rb, while, Cu, Fe, Mo, Se, and Zn were significantly decreased. In plasma, Cu, Mo, Se, and Zn were increased, while As, Cd, Co, Pb, Hg and P were decreased.

L-854

Single molecule fluorescence based approaches to study proteins in cell-like environments

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One of the major goals in biology is to understand the function of proteins and of macromolecular complexes in their cellular context. Fluorescence based methods offer the possibility to measure protein properties and interactions with a high sensitivity and selectivity, even down to single molecule level. In this respect we developed recently approaches to perform quantitative measurements in environments such like crowded solutions. For a quantitative analysis of structures or the dynamics of proteins by single molecule FRET the fluorescence quantum yields have to be known with a high accuracy [1]. However, in studies with proteins under more physiological conditions (e.g. crowded solutions) these parameters are sometimes strongly altered by the surrounding milieu (as compared to pure buffer solutions). To overcome this problem we developed a new method to determine quantum yields of fluorescent dyes attached to proteins at (sub)nanomolar concentrations in these milieus with a confocal microscope [2].
[1] Gabba, M. et al. (2014), *Biophys. J. 107*, 1913-1923.
[2] Kempe, D. et al. (2015), *J. Chem. Phys. B*, 119, 4668-4672

L-855

Magnetic (Torque) tweezers experiments to probe the mechanics and interactions of nucleic acids

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Magnetic Tweezers (MT) can probe single molecules using *magnetic forces and torques*. In the MT, a *superparamagnetic bead* is attached to the molecule of interest, for example double stranded(ds) DNA, while the other end is attached to the bottom of the *flow cell*. By controlling the position and rotation of magnets, above the flow cell, forces (ranging from 0.1 pN to 100 pN) and torques can be applied to stretch and twist the molecules of interest. By varying the magnet configuration different fields can be applied for specific purposes. A "conventional" MT is suitable to analyze the force- extension behavior or to observe DNA supercoiling while rotating the molecules. So called *Magnetic Torque Tweezers* (MTT), offer the opportunity to measure *torque* of dsDNA. Furthermore, our MT setup is able to *track multiple beads* (currently up to ∼20) at the same time, enabling the collection of statistics in a single measurement run.
Currently, we are investigating the precise response of ds DNA to applied forces and torques at varying salt concentrations. Preliminary analysis of force-extension and torque-rotation measurements suggest that the torsional stiffness of DNA does not depend on salt concentration, in contrast to the bending persistence length.

L-856

DNA staining fluorescent proteins for the direct visualization of large DNA molecules

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DNA-binding fluorescent proteins have been utilized for a wide range of bio-applications owing to their ability to directly exhibit protein functions with various colors. Here we introduce a novel design of DNA-binding fluorescent proteins for the staining of DNA molecules. In comparison to the use of fluorescent organic dyes and quantum dots, *in vivo* experiments using FPs are more powerful and versatile due to the possibility of insertion of the FP-tagged gene into a cell or an organism. Alternatively, *in vitro* experiments using FP-tagged proteins have allowed for the study of single molecule protein dynamics on elongated DNA molecules. On the other hand, DNA molecules themselves are usually stained using fluorescent organic dyes such as EtBr, YOYO, SYTO, etc. However, there are certain fundamental drawbacks in the use of these organic dyes in DNA staining. (1) cytotoxicity (2) potential mutagens (3) photodamages. (4) bleaching. Our novel DNA-binding fluorescent proteins have advantages to overcome the limitations of the fluorescent organic dyes. Accordingly, we report the development of a novel DNA-staining FP for the visualization of elongated large DNA molecules within microfluidic devices and nucleoid localization within live bacterial cells.

Abstracts
– 8. Single Molecule Biophysics –

L-857

Intrinsic secondary-structure preferences of amino acids and common post-translational modifications

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The structure and dynamics of a protein is almost exclusively determined by the sequence of its amino acids. Considerable effort has been put into the search for correlations between the physico-chemical properties of amino acids and features such as secondary structure. In this study, we investigate the intrinsic preferences for secondary structure elements of the canonical amino acids and of 47 post-translational modifications (PTMs) commonly observed. We describe extensive molecular dynamics simulations of four distinct frameworks. The results are compared to experimental data and reproduce experimental observables, which include a dominant P_{II} fraction. We also compare different parameter sets of the GROMOS force field to confirm that version 54A7 is appropriate. We observe a broad range of preferences depending on the chemical nature of the side-chains. Moreover, the preferences show similar trends between different frameworks, indicating that context-free intrinsic propensities are a useful concept. To our knowledge, this is the first study including a representative set of modified amino acids, which provides a qualitative prediction of the effects PTMs may have on the amino acids' intrinsic structural preferences, helping to rationalize structural changes induced by them.

L-858

Single molecule blinking behavior of synthetic and biological fluorophores at low temperatures

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Super resolution microscopy methods like STORM rely on the blinking statistics of single dye molecules. Low-temperature experiments have the potential to drastically prolong the survival of the dyes, thus enabling extremely high resolution. Therefore, we investigated the blinking behavior of Alexa Fluor 488 and enhanced green fluorescent protein (EGFP) at 77 K. The samples were vitrified with liquid nitrogen and observed with a confocal microscope. Time traces of individual molecules were recorded, and the blinking behavior was characterized by analyzing the on- and off-times based on an intensity threshold. The distributions followed a power-law in all cases with on/off times ranging from milliseconds to several seconds. The power law exponents ranged from 1.3 to 2. The concentration of cryoprotectant (glycerol or trehalose) showed little influence on the blinking behavior. The survival time of the fluorophores, however, increased with increasing trehalose content. The blinking of EGFP was shown to be pH-independent, in contrast to its behavior at room temperature.
The results of the on/off analysis were consistent with correlation analysis. The latter additionally allowed the determination of triplet kinetics. Surprisingly, EGFP only showed triplet behavior in very few cases.

L-859

Benchmarking single molecule fluorescence instruments: detection in solution

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Single molecule fluorescence microscopy is a frequently used technique for the study of transport mechanisms, signal processing or internal dynamics, giving access to physiological, physical and biochemical parameters inaccessible via ensemble methods. Although single molecule fluorescence detection is widely used, there is a lack of a robust, reliable, and easy to implement method for analyzing and benchmarking these systems.
We have developed a method based on photophysical properties of widely used dyes being capable of determining the excitation rate with its corresponding laser power, which, in conjunction with the detection rate enables for full assessment of the excitation/detection characteristics of the confocal microscope. This method is particularly suited for commercial microscopes, where the excitation power is often given in arbitrary units or just a percentage of the maximum.
We use our method to compare three home-built microscopes in our group under different experimental conditions.

L-860

Pneumatic flow control in microchannels and capillaries

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During the last decade biophysists, biochemists and biologists moved to microfluidics in their applications. Prokaryotic and eukaryotic cells are cultured and investigated in microfluidic chips, they are sorted in microfluidic systems, single molecules are being studied in micro-systems. All of these applications are highly dependent on a precise flow control, which is conventionally done with syringe or peristaltic pumps. However, such approaches have a number of crucial bottlenecks. The regular piston motion of a syringe pump leads to an amplification of the flow velocity typically at ratios of 10^5 in a microchannel. The peristaltic pumps, which use the squeezing of the tubing, do not allow to precisely determine the perfused volume and generate strongly pulsatile flow. We focused on the development and testing of a novel pressure-driven flow control going to the physical limits. We developed a new pneumatic flow control principle without use of a leakage valve for the pressure stabilization in the system (Fütterer et al., *Injection and Flow Control in Microchannels*, Lab Chip, 4, 351, 2004). Our new system overcomes the bottlenecks of all aforementioned conventional flow control methods and demonstrates unprecedented stabilization and fast reaction dynamics.

Abstracts
– 8. Single Molecule Biophysics –

L-861

Copper determined beta-amyloid peptides misfolding: a single molecule assay

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Alzheimer disease (AD) represents a neurodegenerative disorder associated with misfolding of the amyloid peptides (Aβ) and formation of amyloid plaques. The metal hypothesis of AD stipulates that Aβ undergoes a misfolding pathway due to the metal homeostasis thus promoting the formation of neurotoxic Aβ-structures found in patients with AD. Furthermore, histological studies on rats show that although the Aβ peptides are present they do not tend to form deposits and the rats do not show signs of developing AD. The rat Aβ peptide differs from the human one by three mutations point (R5G, Y10F, H13R). Thus, in this study we explore the interaction between truncated Aβ fragments, from human and rat, with copper ions (Cu^{2+}), using single-molecule electrophysiology techniques. By applying a complex kinetic mathematical model we were able to determine the different parameters that describe the interactions between human/rat Aβ1-16 peptides and the α–HL protein, in either free/Cu^{2+}-complexed' form, and reveal the difference in affinity of the Cu^{2+} for the two Aβ1-16 peptides. *Acknowledgements*: IG-2014-08 UAIC, PN-II-ID PCCE-2011-2-0027; PN II-PT–PCCA-2011-3.1.0595 Nr. 123/2012.

L-862

The mechanism of pre-initiation compex driven promoter opening and transcription start site scanning

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Transcription initiation in Eukaryotes depends on the formation of the formation of the multi-protein complex known as the Pre-Initiation Complex (PIC). In *S. cerevisiae*, transcription initiation on TATA-dependent promoters has at least four phases. First, PIC-formation is nucleated by the binding of TATA-box binding protein (TBP) to the TATA-box element on the promoter. Second, the XPB subunit of TFIIH catalyzes DNA unwinding and establishes a DNA bubble. Third, the PIC performs transcription-start-site scanning (TSS scanning) where DNA sequences downstream of the initial site of DNA opening are surveyed for potential initiation sites. Lastly, RNAP II escapes the PIC and enters into processive transcription initiation. The mechanism of DNA opening and subsequent transcription-start-site scanning is unknown. Here, using single-molecule magnetic tweezers approaches, we measure the distributions of DNA bubble size and lifetime formed in the presence of reconstituted PICs with varying ATP and NTP concentrations on both negatively and positively supercoiled DNA. Our data allow us to place constraints on the mechanism of transcription initiation in Eukaryotes and serve as a foundation for future single-molecule studies of this complex and critical step in eukaryotic transcription regulation.

L-863

Probe the dynamics of nucleic acids and proteins with high frequency Magnetic Tweezers

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Magnetic Tweezers (MT) are a single molecule technique that enables the application of both forces and torques to biological macromolecules such as DNA molecules or proteins. The molecules are attached with one end to superparamagnetic beads, while their opposite ends are attached to the bottom surface of a flow cell. Magnets, placed above the flowcell, exert magnetic fields such that a force clamp is applied over a long period of time, without the need for feedback.
Camera-based tracking is used to monitor (x,y,z)-position of the beads. Recent improvements in CMOS technology make it possible to track many beads at the same time, enabling us to perform multiple (currently up to ∼200) single molecule measurements in parallel to address biological challenges with large statistical data sets. Alternatively, a reduced field of view can be used which enables fast measurements with frames rates in the kHz regime.
Here, we present high frequency magnetic tweezers measurements on dsDNA molecules. Using the instrument in kHz-tracking mode, we are investigating the buckling transition of dsDNA as a function of force and different salt concentrations. Preliminary analyses suggest an increase of buckling time with increase of force, as well as a decrease of buckling time with decrease of salt.

L-864

Why are biological systems nonlinear?

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Physical systems can be studied using either classical or quantum physics. It is of equal importance to choose between linear and nonlinear physics.
Biophysical systems are essentially nonlinear due to weak interactions. Two examples are explained. The first is DNA molecule. Nucleotides belonging to different strands interact through hydrogen bond. This interaction is weak and is usually modeled using Morse potential. This function is nonlinear and, consequently, yields to nonlinear differential equation.
The second example is microtubule, which is an essential part of cytoskeleton. The weak interaction is coming from W-potential, describing influence of surrounding dimers on the particular one. This potential is also nonlinear and, like above, brings about nonlinear differential equations, describing nonlinear dynamics of microtubules.
Generally speaking, linear interactions are valid only for strong forces between neighbouring particles. This means that their displacements are small and we can assume that attractive and repulsive forces are equal. However, for weak interactions these displacements are larger and we cannot equate attractive and repulsive forces any more. To describe these weak interactions only nonlinear potentials are acceptable.

Abstracts

– 9. Material Science in Biophysics –

L-865

Label free detection of ESCRT-III binding on black lipid membranes through a novel LSPR approach

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The endosomal sorting complexes required for transport-III (ESCRT-III) family of proteins catalyses membrane fission necessary for a myriad of cellular activities including cytokinesis and budding of some enveloped viruses like HIV-1 among others. In order to measure ESCRT-III binding to membranes appropriate model bilayers and read-out systems are required. Given the free standing nature, amenability to solute exchange on either side of the membrane and optical imaging, black lipid membranes (BLMs) form ideal membrane-analogue for the ESCRT-III. Localized surface plasmon resonance (LSPR) offers powerful means for sensitive label-free detection of protein-membrane and protein-protein interactions in a highly multiplexed format. Here we attempted to exploit these features by developing an LSPR assay for label free detection of ESCRT-III binding to BLMs. Gold nanoparticles are targeted to the BLM through specific molecular interactions, followed by binding of proteins to the membrane. The interaction is monitored with an epi-illumination system, permitting LSPR measurement over a fixed wavelength range. Upon protein binding, changes in the LSPR signal due to red-shift are recorded.
The discussed methodology would be extended to other peripheral and integral membrane proteins.

L-866

Interplay between photodymanic oxidation of lipids and elastic properties of lipid bilayer

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Elastic constants of lipid bilayer are key parameters that control various functions, which cellular membranes perform. Abnormal changes of elastic properties of membranes could lead to sever cellular dysfunctions. Pathological processes associated with interaction of lipids and active radicals (singlet oxygen) could lead to pronounce change of chemical composition and induce membrane breakage. Such significant alterations of lipid composition should modify mechanical properties of membrane. However, it remains unknown how elastic properties of lipid bilayer change upon lipid oxidation. In this study we investigate the influence of photodynamic lipid oxidation on membrane bending rigidity. We use Rudin-Muller membrane as a model of the lipid bilayer and unsaturated fatty acids as the targets for the singlet oxygen produced by photosensitizers. We show that notable changes of bending modulus and spontaneous curvature of lipids always precede the loss of membrane integrity. The obtained data allowed us to establish relation between elastic properties of the bilayer and changes of molecular geometry of lipids upon oxidation.

L-867

On-chip integrated glass microtubes for studying 3D stem cell migration in vitro

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Mammalian cells in tissues are constantly in contact with their intricate 3D environment. The extracellular matrix (ECM) provides complex local chemical and topographical information, which regulates and fine-tunes cell behavior. Thus, efforts are undertaken to develop *in vitro* scaffolds that mimic aspects of 3D ECM and therefore possess better biological or clinical relevance. However, studies employing 3D microarchitectures so far comprise the modification of several scaffold parameters at once. Here, our tubular microtubes present a well-defined *in vitro* scaffold for the study of dimensionality and variable confinement on cell migration. When culturing neural stem cells in biofunctionalized SiO/SiO_2 rolled-up nanomembranes we monitored a distinct cellular phenotype that, mediated by the microtube confinement, resembled better the morphology of cells in the developing brain. Additionally, the migration phenotype linked to the dimensionality of the microtube scaffold suggested a mesenchymal to amoeboid migration mode transition. Our study stresses the impact of scaffold dimensionality on the cell response and establishes the rolled-up tubular structures as an *in vitro* stem cell culture system that emulates topographical information and space restriction present in dense tissue.

L-868

Capturing the complex kinetics of lipid membrane pore formation by Force-Clamp Spectroscopy

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Cell membranes must sustain a wide range of forces in vivo and therefore their physical integrity is fundamental for cell survival. Large scale rupture of lipid membranes has been suggested to initiate with the formation of an individual nano-sized pore[1]. Hence, the mechanism of pore formation might be key for the whole physical integrity of the lipid membrane. We have applied Force-Clamp Spectroscopy with Atomic Force Microscopy (AFM) to study the complex kinetics of rupture in stacks composed of tens of supported lipid membranes, thus avoiding any possible substrate effect. Our experimental setup allowed for the first time capturing intermediate steps in the rupturing process of lipid membranes. The observed intermediates can be assigned to the rupture of the two separate leaflets and the inter-membrane water layers. Among these, the rupture of the (upper) leaflet is the determinant step of lipid membrane rupture kinetics. Furthermore, its rupture time distribution is in agreement with a model of pore nucleation. Therefore, our results indicate that the rupture of a lipid bilayer should be regarded as a complex nucleation process with several intermediate steps instead of the current all-or-none, simple two state process.
[1]M Kotulska, Biophys J, (2007) Is. 92, V. 7, 2412-242

Abstracts
– 10. Protein-Nucleic Acid Interactions –

L-869

Acquisition of a novel RNA binding mode relying on extended dsRBD for cooperative post-transcriptional modification in human tRNAs

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tRNAs are subject to post-transcriptional base modification during their maturation. Besides ensuring translation fidelity and efficiency, these modifications are essential for tRNA stability, preventing them from rapid degradation by RNAses. The extent of tRNA modification varies depending on cell metabolism. For instance, large amounts of dihydrouridine are produced in certain human cancers. This is related to a high level of expression of dihydrouridine synthase 2 (HsDus2), which promotes cell survival through interactions with the pro-apoptotic proteins PKR and PACT. In contrast to its paralogs, HsDus2 acquired a new tRNA recognition mode relying on an unusual double stranded RNA binding domain. Our crystal structures, biochemical and yeast complementation analysis illustrate this new tRNA binding mode for cooperative dihydrouridine synthesis.

L-870

Recognition and condensation of the bacterial centromere by ParB

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In *B. subtilis* chromosome segregation is a multipartite task shared by the *ParABS* system and the Structural Maintenance of Chromosomes (SMC) condensin complex. At the most basic level of this hierarchal assembly, ParB binds at and around *parS* sequences, locally condensing DNA and acting as a positional marker of the replication origin. This complex is a cargo for ParA-mediated segregation and a loading site for condensin. However, the mechanisms of ParB assembly at *parS* and the ensuing segregation and condensation of the chromosome are poorly understood. In previous work we suggested a model for ParB association and condensation around *parS* involving distinct specific and non-specific DNA binding loci. Here we test this model by assessing the properties of mutant ParB proteins using biochemical and biophysical assays. Mutation of a central helix-turn-helix region completely eliminates specific binding to *parS* sequences, but non-specific DNA binding and condensation are largely unaffected. Moreover, we provide direct evidence for a second DNA binding locus in the C-terminal domain of ParB and show this region is also important for oligomerisation leading to DNA condensation. A refined model for ParB-DNA complexes at the bacterial origin is discussed.

L-871

The effect of purine 2-amino group on the DNA groove width of FIS-DNA complex

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The width of the DNA minor groove varies with sequence and can be a major determinant of DNA shape recognition by proteins. For example, the minor groove within the center of the Fis-DNA complex narrows to about half the mean minor groove width of canonical B-form DNA to fit onto the protein surface. G/C base pairs within this segment, which is not contacted by the Fis protein, reduces binding affinities up to 2000-fold over A/T-rich sequences. We show here through computational methodology that the 2-amino group on guanine is the primary molecular determinant controlling minor groove widths. Molecular dynamics simulations of free-DNA targets with canonical and modified bases demonstrate that sequence-dependent narrowing of minor groove widths is modulated almost entirely by the presence of purine 2-amino groups.

L-872

NPDock – a web server for protein-nucleic acid docking

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Protein-RNA and protein-DNA interactions play fundamental roles in many biological processes. A detailed understanding of these interactions requires knowledge about protein-nucleic acid complex structures. Because the experimental determination of these complexes is time-consuming and perhaps futile in some instances, we have focused on computational docking methods starting from the separate structures. Docking methods are widely employed to study protein-protein interactions; however, only a few methods have been made available to model protein-nucleic acid complexes. Here, we describe NPDock (Nucleic acid – Protein Docking); a novel web server for predicting complexes of protein-nucleic acid structures that implements a computational workflow that includes docking, scoring of poses, clustering of the best-scored models, and refinement of the most promising solutions. The NPDock server provides a user-friendly interface and 3D visualization of the results. The smallest set of input data consists of a protein structure and a DNA or RNA structure in PDB format. Advanced options are available to control specific details of the docking process and obtain intermediate results. The web server is available at `http://genesilico.pl/NPDock`.
This website is free and open to all users and there is no login requirement.

Abstracts

– 11. New Methods for Computational Biophysics –

L-873

Simulating cell migration using subcellular element approximations

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All intercellular and intracellular processes who play role in migration finally lead to mechanical procedures that control the dynamics of cells. A detailed study of the inner constituents of cells in a multicellular environment seems to be impossible due to current computational capacity. However, recently specific methods such as Subcellular Element Model (SEM) and Cellular Particle Dynamics (CPD) have been introduced to simulate the behavior of a large number of cells by means of mechanical properties.
In the current study, we propose a model for simulating cell behavior both in single cell case and multicellular systems. The aforementioned behavior is affected by the core constituents of the cells and the interactions between them. We can switch from single cell to multi cell simulation by decreasing the details we expect from the simulation. In this regard, in our model we use cells representing the single-cell mechanical properties in a semi-quantitative manner. The effect of cell stiffness on cell migration is studied. Our results showed that less stiff cells are more motile while cell-ECM adhesion also affects motility. Last but not least, we studied how the ECM stiffness could affect the migration of a cell from the mechanical point of view.

L-874

Kinetics and modeling of diphtheria toxin (FA) and actin interaction

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Diphtheria toxin (DTx) is separated into two fragments after limited proteolytic digestion. Fragment A (FA) (21 kDa) has an enzymatic activity (ADP-ribosyltransferase) at the end of the N-terminal that catalyses the transfer of an ADP-ribosyl group of NAD to a post-translationally modified histidine (diphthamide) residue on eukaryotic elongation factor 2 (eEF2). Fragment B (FB) (39 kDa) that provides the connection between the cell and holotoxin. Actin, is the main component of the microfilament structure in eukaryotic cells. Moreover, it interacts with a group of proteins and acts as a junction in the signal pathways. For this hence, it was shown that DTx causes actin depolymerization. In this study, it was shown by gel filtration and viscosity measurements that FA can interact with both G (globular) and F (filamentous) actin from the positive end. This interaction was inhibited by gelsolin or DNase I. Additevely, FA and G-actin were obtained with homology modelling and interacted with (NAMD, Docking) methods. The possible interaction site with high binding energy, was determined with the Maestro program that was devoloped by Max-Planck Instute.

L-948

FMM with lambda-dynamics support on GPUs

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A realistic description of electrostatic interactions in (bio)molecular simulations has to take into account that the molecules possess titratable sites which charge distribution varies.
Uptake and release of protons or tautomerism are the most ubiquitous causes of this variability. Other examples include oxidation and reduction reactions or binding and release of metal ions and other small-molecule ligands.
In the GromEx project, we are combining a fast multipole method (FMM) with lambda-dynamics for an efficient and at the same time realistic computation of of electrostatic interactions. The combined method will first be available in the popular GROMACS simulation package.
The FMM aspect of the combined method allows for a highly scalable parallel computation of electrostatic interactions while naturally allowing for alternative, local charge distributions of titratable sites.
Taking full advantage of the immense compute power of graphics processing units (GPUs) is an important goal of our development work. Meeting this aim poses especially high demands on the parallelization of the method in terms of the degree of parallelism and in terms of optimally matching hardware and algorithmic requirements.

Abstracts
– 12. Biospectroscopy, EPR and NMR –

L-875

Use of Raman spectroscopy to determine cardioprotection by NO donor compounds

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Several studies have shown that nitric oxide donors protect cardiomyocytes against ischemia and reperfusion. However, until present, the mechanism of the cardioprotective effect of nitric oxide donor in ventricular cardiomyocytes is not fully understood. In this research, pre-resonance Raman spectroscopy was applied to investigate the mechanism of cardioprotection by nitric oxide donors in isolated cells exposed to metabolic inhibition and re-energisation. Metabolic inhibition and reenergisation were used in this study to mimic the low and high oxygen levels experienced by cells during ischaemic and reperfusion treatments. The results demonstrate that the technique has the capability to evaluate whether or not a pre-treated cardiomyocyte has been effectively protected prior to exposure to the metabolic inhibitor. A laser wavelength of 488 nm used in this study has been found to provide the most sensitive means of observe the cellular mechanisms of myoglobin during nitric oxide donor preconditioning, metabolic inhibition and re-energisation and did not cause any damage to the cells.

L-876

EPR measurements of nanometer distances in RNA and peptides in lipid bilayers

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Double electron-electron resonance (DEER/PELDOR) is an EPR method to detect weak dipole-dipole interactions between paramagnetic centres. Distance measurements with DEER in combination with site-directed spin labeling can provide information on interactions and rearrangements of biological units in bio-macromolecules. The quality of the information depends on the capability of the label to report on the molecular structure without affecting it. A correlation of the experimental distance to the structure of the biomolecule might be best achieved with labels of reduced intrinsic mobility. RNA was labeled with a TEMPO-based cytosine spin label that preserves the W–C base-pairing capability of the labeled nucleotide.[1] A systematic change in label positions in a ruler like fashion results in narrow distance distributions from 3–6 nm, thereby highlighting the potential of the label for high accuracy distance measurements. A study on model transmembrane peptide WALP24 using most commonly used flexible nitroxide label MTSSL as well as the rigid TOPP[2] demonstrates the ability of DEER to extract distance information within peptides incorporated into different lipid bilayers.

1. G. Sicoli et al, *Angew. Chemie Int. Ed.*, **2010**, 49(36)
2. S. Stoller et al, *Angew. Chemie Int. Ed.* **2011**, 50(41)

L-877

Characterisation of Protein-Ligand Interactions Using Solution-State NMR spectroscopy

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Carboxypeptidase G2 (CPG2) is an enzyme that cleaves C-terminal glutamate from an extensive range of N-acyl groups. CPG2 is currently exploited in cancer therapy: following its administration as an immunoconjugate, in which CPG2 is attached to an antibody directed at a tumour associated antigen, it can enzymatically convert inactive pro-drug to a cytotoxic drug selectively at cancer sites. However, preclinical experiments concluded that an intermediate step involving inhibition of CPG2 from the circulatory system before pro-drug administration would be indispensable to prevent systemic toxicity. Accordingly, work had started making peptide-based ligands directed at the enzyme CPG2. Due to its therapeutic applications in the treatment of cancer and autoimmune diseases, the three-dimensional structure of CPG2 is of great interest. Hence, the ambition is to better understand the mode of binding of these ligands by acquiring structural and thermodynamic data of the free enzyme and enzyme-ligand complex using high-field solution-state nuclear magnetic resonance (NMR) spectroscopy and other biophysical techniques (MALDI-TOF, UV-visible spectroscopy, CD and X-ray crystallography). The information gained could then be used to design new and ameliorated peptide-based ligands.

L-878

Binding scheme between 14-3-3ζ and phospho-TH elucidated by NMR and computational studies

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Our recent ^{31}P NMR data showed much more complex binding mode between 14-3-3ζ and doubly phosphorylated peptide of human tyrosine hydroxylase 1 (hTh1) than was originally thought (Hritz et al. *Biophys J.* **2014**, 107, 2185). Analysis of the binding data revealed that the 14-3-3ζ dimer and the S19- and S40-doubly phosphorylated hTh1 peptide interact in multiple ways, with three major complexes formed: (1) a single peptide bound to a 14-3-3ζ dimer via the S19 phosphate with the S40 phosphate occupying the other binding site; (2) a single peptide bound to a 14-3-3ζ dimer via the S19 phosphorous with the S40 free in solution; or (3) a 14-3-3ζ dimer with two peptides bound via the S19 phosphorous to each binding site.
Experimental determination of the binding affinities and binding modes between 14-3-3ζ dimer and their phosphorylated protein partners is very tedious therefore we have decided to address this problem also by computational techniques. Binding/unbinding pathways and the corresponding absolute binding affinities of the selected phosphopeptides with respect to the 14-3-3ζ have been studied by Hamiltonian Replica Exchange Molecular Dynamics (H-REMD) combined with a novel reaction coordinate approach (distancefield), and potential-of-mean-force (PMF) methods.

Abstracts
– 12. Biospectroscopy, EPR and NMR –

L-879

Measuring intra-molecular distances by anomalous small-angle X-ray scattering

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Measurements of molecular distances are key to dissect the structure, dynamics, and functions of biological macromolecules. While FRET and NMR-based techniques have provided invaluable details by measuring intra-molecular distances, they suffer from a limited range (< 10 nm) and difficulties in converting the measured signal into absolute distances. SAXS measurements employing gold-nanoclusters as labels on DNA constructs have demonstrated their ability to provide information about the entire gold label-gold label distance distribution for a considerable range of distances. Here, we demonstrate measurement of intra-molecular distances on 10, 20, and 30 bp DNA constructs carrying two small (∼1 nm) gold labels using anomalous small-angle X-ray scattering (ASAXS). Our approach only requires the double-labeled samples and relies on recording scattering profiles for each sample at different energies. By tuning the X-ray energy through the gold L-III edge (at ∼11.9 keV), it is possible to separate out the gold contributions from the DNA only and gold-DNA scattering term. Our results demonstrate that ASAXS based determination of label-label distances is possible and provides an attractive alternative to determine absolute intra-molecular distance distributions.

Abstracts

– 13. Biomolecular Simulation and Computational Biophysics –

L-880

Integrating RDC data into simulated IDPs ensembles

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Instrinsically Disordered Proteins (IDPs) are a challenge to structural biology for several reasons. They question the sequence-structure-function paradigm, and are difficult to study experimentally. NMR spectra measure the average properties of an ensemble of structures, which can be obtained from different conformational ensembles. Thus simulations play a more relevant role than for folded proteins. At the same time, they are more error prone due to the subtle energy landscapes of these proteins. In this poster we explain the use of the Maximum Entropy principle to bias simulations to fit NMR data, and we show a tool (ss-map) to visualize the length of the secondary structure elements in IDPs ensembles.

L-881

Folding of amphiphilic peptides at macroscopic and molecular interfaces

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The conformational preference and thus the biological functions of peptides depend on environmental conditions. Therefore, understanding the driving forces behind environment induced conformational changes such as transition from a disordered state to an alpha-helix or beta-sheet is essential. Using molecular dynamics simulations we investigate the conformational behavior of two synthetic peptides, LK and EALA, with built-in secondary amphiphilicity favoring an alpha-helix. Although the peptides are designed to adopt alpha helices, we observe that they do not have a preferred unique secondary structure and adopt a variety of different conformations in solution at infinite dilution. Exposure to a hydrophobic/hydrophilic interface such as an air/water interface triggers partitioning of hydrophobic and hydrophilic residues initiating folding into alpha-helices as the hydrophobic periodicities of these peptides suggest.
Alternatively, the neighboring peptides can act as molecular interfaces and influence folding similar to the macromolecular one. When macroscopic partitioning is coupled with aggregation, the balance of opposing forces of electrostatic repulsion, hydrophobic attraction and intra- and intermolecular hydrogen bonding, determines aggregate stability and structure.

L-882

The dynamic difference between HCV NS3/4A protease from genotypes 1b and 4a: A computational study

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Hepatitis C virus infection is a primary cause of chronic hepatitis and leads eventually to liver cancer. Egypt has the largest infections worldwide. The most dominant genotype in Egypt is 4a. HCV NS3/4A is a primary target of designed antivirals. Few structural and dynamic studies of HCV NS3/4A protease from genotype 4a have been done to infer the difference in dynamics, enzymatic activity and substrate binding between it and other widely studied genotypes such as 1b. Most of the designed drugs for genotypes 1a and 1b confer drug resistance with 4a. A homology model of genotype 4a has been built with bound NS5A/5B substrate. The dynamic differences and substrate binding between 4a and 1b have been studied using MD simulations. It is found that 4a has higher conformational flexibility (Larger RMSDs) than 1b, but 1b has higher root mean square fluctuations than 4a. The substrate binding has different impact on RMSF of 4a annd 1b indicating different interactions. The electrostatic interactions (salt bridges and H-bonding pattern) show differences between the two genotypes. The catalytic residues of 4a have higher RMSDs than 1b which confer higher structural instability affecting the hydrolysis mechanism and function.

L-883

Protein Complexes from MD Simulations: the Rope-Pulling Game of Tapasin and MHC I Molecules

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We present molecular dynamics (MD) simulations of the association between tapasin (Tsn) and major histocompatibility complex class I (MHC I) molecules, two key players of the antigen-processing pathway. Due to the lack of structural data on the protein complex, the molecular events surrounding peptide loading onto MHC I were not well understood. We show that the selection of high-affinity antigens from the pool of mostly low-affinity peptides is the result of a molecular game of rope-pulling: Tsn pulls on a region of the peptide binding groove of MHC I to open it, whilst the peptide simultaneously tries to close the groove. Low-affinity peptides lose the challenge and are exchanged until a high-affinity contender closes the groove, thereby initiating complex breakdown. Our simulations also explain the chaperone action of Tsn on peptide-deficient MHC I. Protein complexes are crucial for cellular processes, yet often very little is known about their structural organisation. Due to the large system sizes and time scales, theoretical studies of protein-protein association usually employ approximations that do not take into account protein dynamics. Here, we employed all-atom MD simulations in explicit solvent to study protein-protein association on a multi-microsecond time scale.

Abstracts

– 13. Biomolecular Simulation and Computational Biophysics –

L-884

Molecular simulations shed light on mechanisms of cooperative DNA recognition

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In pluripotent cells, the transcription factors OCT4 and SOX2 bind cooperatively to composite DNA elements to regulate gene expression. OCT4 belongs to the POU family, hence it has two DNA binding domains, a POU specific (POU_S) and a homeodomain (POU_{HD}) connected by a flexible linker. The direct interaction with SOX2 involves only the POU_S. From unbiased simulations of OCT4-DNA and OCT4-SOX2-DNA complexes we found that SOX2 modifies the orientation and dynamics of the DNA-bound configuration of OCT4. This involves the remodeling of the POU_{HD}-DNA interaction despite the lack of direct contacts between SOX2 and the POU_{HD}. Remarkably, the DNA mediates the SOX2-POU_{HD} communication. From simulations of protein-DNA dissociation we found that SOX2 modifies the unbinding profiles of both OCT4 domains. Thus, we demonstrate that the OCT4-SOX2 cooperativity is modulated by an interplay between protein-protein interactions and DNA-mediated allostery. Finally, we accurately estimated the cooperativity and found that SOX2 affects the relative DNA-binding strength of the OCT4 domains, hence modifying their roles in exploring the DNA. These findings contribute to the establishment of the mechanisms of DNA recognition by multidomain transcription factors and regulation of cellular pluripotency.

L-885

MD simulation studies of a bilayer built of *E. coli* lipid A

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Lipid A is regarded as the most chemically invariant part of lipopolysaccharide (LPS), which is the major component of the outer membrane of Gram-negative bacteria. *E. coli*-specific hexa-acyl lipid-A (ECLA) forms stable bilayers in the presence of either sodium or magnesium cations, and its main phase transition temperature is ∼317K. Here, we report 800-ns molecular dynamics simulations of fully hydrated ECLA bilayers. The bilayers were validated against scarce experimental data including surface area per lipid, the membrane width, and known conformations of the beta 1-6 glycosidic linkage in carbohydrates showing structural similarity to the diglucosamine part of lipid A. Our study reveals strong inter-lipid links and numerous H-bonds with water in the interfacial region of the ECLA bilayer. At the interface, also sodium ions are readily bound to the polar groups of ECLA. Altogether, the lipid A bilayer emerges as a solid barrier, which provides a bacterial cell with reliable protection against hazardous environmental factors. These protective features of the lipid A bilayer can only be strengthened by the presence of densely packed long polysaccharide chains of LPS in the outer bacterial membranes.

L-886

Energetic and topological origins of phosphorylation induced disorder-to-order transition

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Recent work on intrinsically disordered proteins points to a unique mechanism wherein phosphorylation of a functional disordered protein 4E-BP2 induces a conformational switch to a non-functional ordered state. Here, we explore the origins of this unique mechanism employing a structure-based statistical mechanical WSME model. We find that the model is able to reproduce the changes in stability upon different degrees of phosphorylation. The predicted one-dimensional free energy profile and two-dimensional landscapes indicates that in the dephosphorylated state, the protein samples multiple conformations and the equilibrium gradually shifts towards the fully folded state upon phosphorylation. We further map out the possible folding mechanism of 4E-BP2 and identify that a two-dimensional landscape is necessary to account for the folding complexity with at least 3 states that includes an off-pathway intermediate. A large-scale *in silico* mutational analysis of charged residues convincingly shows that the folded state is electrostatically optimized for stability while being simultaneously non-optimal from topological considerations. Finally, we show that the folding and functional requirements are intertwined in 4E-BP2 effectively determining the complex conformational behavior.

L-887

Theoretical research related to the concentration dependency of peptide aggregation

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Proteins are biopolymers that are composed of 20 kinds of amino acids, and they usually fold into their native structures in order to perform their biological functions. However, they sometimes fold into incorrect structures, and this event is referred to as "misfolding". There are some diseases caused by misfolding, and these diseases are named "folding diseases". Alzheimer's disease, which is our research target, is also a kind of folding disease. It is well-known that Alzheimer's disease is caused by misfolding of the amyloid-beta peptides. According to some hypothesis, the cause of Alzheimer's disease is misfolding into beta-sheet structures of amyloid-beta peptides, and forming insoluble fibrous proteins called "amyloid fibrils".
Recently, an interesting report about the mechanisms of the amyloid fibril formations has been published. They claimed that the concentration is important for the self-assembly of amyloid fibrils. In order to examine the concentration dependency of the amyloid peptide aggregation theoretically, we performed molecular dynamics simulations. We prepared a system consisting of 8 fragments of amyloid-beta (25-35) and applied the replica-exchange molecular dynamics method in order to examine the concentration dependency.

Abstracts

– 13. Biomolecular Simulation and Computational Biophysics –

L-888

Free energy landscapes and the unfolding motion of a 7-bp DNA by molecular dynamics simulations

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The formation of specific DNA secondary and tertiary structures has been reported to play a key role in various range of biological processes, such as transcription termination or intermolecular binding. Among them, a pivotal role has been ascribed to DNA i-Motif and G-Quadruplex structures, which due to their biological appearance in telomeric and centromeric DNA are considered as potential targets for various diseases. Recent studies on high-temperature unfolding simulations of the DNA i-Motifs have revealed the existence of stable hairpin configurations as an intermediate step in the unfolding pathway of DNA higher-order structures. In our study, we investigate a simple 7-nucleotide DNA hairpin structure with the sequence d(GCGAAGC) to get insight into the stability of DNA hairpin structures in more detail. The resulting free energy landscape has been calculated via Metadynamics. Together with high-temperature molecular dynamics simulations, this allows us to get insight into the stability of the DNA structures with respect to the estimated free energy barriers. This serves as the first approach to unravel the complex nature of DNA higher-order structures folding pathway and behavior.

L-889

Internal allosteric sodium in the δ-opioid receptor responds to transmembrane voltage

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G-protein-coupled receptors (GPCRs) are the largest superfamily of membrane proteins within the human genome. They participate in numerous physiological functions, including neuronal excitability and pain signalling. Owing to their functional and structural characteristics, they are excellent drug targets. In spite of their diversity, it is thought that GPCRs share a conserved pathway of signal transduction via conformational changes in their transmembrane (TM) domain. The full range of movements leading to activation, and their interaction with external factors, are however still incompletely understood. Many GPCRs are for instance modulated by sodium. The recent high-resolution crystal structure of the delta-opioid receptor (DOR) provides detailed insight into the sodium binding site in the core of the TM domain. In this work, we looked at the effect of sodium ions and transmembrane voltage on the flexibility and conformational changes of DORs. We investigated the structure of DOR in double-bilayer, atomistic simulation systems under physiological and supra-physiological transmembrane electric fields applied by CompEL, to characterise the role of sodium in DOR. Our results implicate sodium and voltage as key players in controlling the conformation and function of the δ-OR.

L-890

Infrared spectra of small-molecule oligomers computed with molecular dynamics simulations

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Many molecules show a different signature in their vibrational spectra, depending on whether they are in a monomeric state, clustered to oligomers via e.g. hydrogen bonds, or form short covalently bonded polymers. In addition to the obvious shift or disappearance of bands that can be assigned to vibrations of the atomic groups that are directly involved in such bonds, secondary effects can also be observed on e.g. neighbouring groups. We present here an analysis of the changes in vibrational signature upon oligomerisation from a combination of classical molecular dynamics simulations, allowing to explore distributions of cluster size and life times, with first principles simulation of infrared-spectra in solution at finite temperature.

Abstracts

– 14. Systems Biology and Multi-Cellular Systems –

L-891

Excitability of neuronal networks interconnected by a 16-state gating model of gap junction channels

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Gap junction (GJ) channels formed of connexin (Cx) proteins are responsible for cell-cell communication. We combined Hodgkin-Huxley formalism with a stochastic 16-state model (S16SM) of GJs gating to examine synchronization of neuronal networks and the spread of excitation. A S16SM describes the probabilistic behavior of *fast* and *slow* gates arranged in series in each of two hemichannels of the GJ channel. Voltage-gating of the GJ channel depends on unitary conductances and I-V rectification of gates and a fraction of transjunctional voltage (V_j) that falls across each of two *fast* and two *slow* gates. The model allowed us to evaluate dynamic changes of junctional conductance (g_j) at macroscopic and single channel levels depending on V_js arising during asynchronous excitation of neighboring neurons. We demonstrate that g_j can be affected in Cx-type dependent fashion by V_j-gating during a bursting activity of neurons and by I-V rectification of *fast* and *slow* gates. We validated conditions under which in a 2-D cluster of neurons develop unidirectional signal transfer and reverberation of excitation, which is one of forms of short-term memory and its consolidation into long-term memory.

L-892

Differences in photophobic response between euglena gracilis and chlamydomonas reinhardtii

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We found that the photophobic response of Euglena gracilis differs from that of Chlamydomonas reinhardtii, although the two green algae are very close in photosynthesis and motility with flagellum. We examined the irradiation of patterned blue light onto the cell populations of E. gracilis and C. reinhardtii, separately confined in 2D microchambers with a depth of 150 um, and evaluated their swimming movements by real-time trace extraction from live images. The cells of E. gracilis migrated out from the illuminated area, whereas those of C. reinhardtii were activated more in the illuminated area. The difference is attributed to their swimming direction changes when exposed to strong light, i.e., E. gracilis randomly changes its swimming direction, whereas C. reinhardtii detects the light direction and swims to the counter direction. The probable hypothesis is that rhodamine, the photoreceptor of C. reinhardtii, produces chemical redox gradient in C. reinhardtii cell body, which selects one of the three states of swimming direction as right/straight/left. In contrast, photoactivated adenylyl cyclase, the photoreceptor of E. gracilis, just switches two states of swimming direction as straight/turn.

L-893

Scaling and regeneration of self-organized patterns

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Biological patterns and morphologies, generated during development and regeneration, often scale with organism size. Some animals such as flatworms can even regenerate a miniature version of themselves from a tiny amputation fragment. Moreover, flatworms scale their body plan up and down depending on feeding conditions.
Inspired by these examples, we propose a generic theoretical mechanism that spontaneously generates patterns that scale with system size. We study a minimal system, in which we couple a Turing instability to the reaction kinetics of diffusing expander molecules, which regulate the reaction rates of the Turing system in turn. Thereby, the expander adjusts the pattern length scales proportional to system size.
Turing mechanisms do not naturally scale with system size. Instead, the same pattern is repeated in larger systems. In contrast, our generalization of Turing patterning is both self-organized and self-scaling. Using dynamical systems theory, we identify minimal requirements for scaling as well as experimentally testable signatures of pattern regeneration. Thus, our model captures essential features of body plan regeneration in flatworms as observed in experiments.
See also: Werner et al., Phys. Rev. Lett. 114, 138101 (2015) (Editor's suggestion)

Abstracts
– 15. Biologically Active Peptides –

L-894

MD study on antimicrobial peptaibol harzianin HK VI

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Harzianin HK VI is an antimicrobial peptide (AMP) of the peptaibol family, like the extensively studied alamethicin (Alm). Peptaibols are characterized by the presence of an unusual amino acid, α-aminoisobutyril acid (Aib), a C-terminal 1,2-aminoalcohol and an acetylated N-terminus.
Harzianin HK VI is biosynthesized by the fungus Thricoderma pseudokoningii. It comprises only 11 aminoacids but despite the short sequence it exhibited noticeable membrane activity [1].
The mechanism of its interaction with the microbial membrane is unknown at present.
The aim of our study is to characterize the conformation of the harzianin HK VI molecule as well as its orientation in the membrane. We perform MD simulation of harzianin HK VI inserted into a DMPC bilayer.
A further work will involve free energy simulations for processes like insertion of harzianin into the membrane, change of orientation, conformation transition and association of two or more harzianin molecules.
[1] Rebuffat, S. et al, *J. Chem. Soc., Perkin Trans. 1,* 16 (1996) Pages 2021-2027.

L-895

Structural parameters for fluorine-labeled helical peptides

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Fluorine labeled amino acids (^{19}F labels) are an excellent tool to study the properties of antimicrobial peptides in oriented lipid bilayers by solid-state NMR. The high sensitivity and the absence of background signals in ^{19}F NMR allows the use of smaller amounts of peptides and shorter measurement times than with ^{2}H, ^{15}N or ^{13}C SSNMR. Because of the artificial character of the ^{19}F labels, strict design principles and experimental verification are necessary to prove that these labels do not disturb the peptide structure or activity.
The orientational constraints obtained by the use of intact Ala-d_3 labeled peptides are very similar to those obtained by the use of the ^{19}F labels. So far, ^{19}F labels have been used only to replace hydrophobic amino acids. In order to use fluorine labeled amino acids also as replacement for other amino acids, a range of ^{19}F labels has been designed, which are already existing or under current development. Here, a computational study is presented, where different ^{19}F labels are incorporated into the well-studied antimicrobial peptide PGLa. The MD simulations show that the general peptide structure and orientation is not affected by the labels.

L-896

Selective membrane poration by the anticancer peptide killerFLIP-E: role of charge and aggregation

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*Killer*FLIP-E is a peptide originally designed to interfere with apoptosis signalling. While it does exhibit a strong anticancer activity and low toxicity, it induces an apoptosis-independent cell death, accompanied by loss of membrane integrity [1]. Based on this observation and on the sequence similarity between *killer*FLIP-E and antimicrobial peptides [2], a mechanism of action related to membrane perturbation was proposed. To test this hypothesis, we studied peptide interaction with liposomes. The peptide did cause leakage of vesicle contents, but only in membranes containing anionic lipids, which are present on the external surface of cancer cells. This selectivity was due to a higher affinity of the cationic peptide for charged than for neutral bilayers. In addition, light scattering experiments showed that this amphipathic peptide aggregates for [*killer*FLIP-E]>0.5 μM, thus reducing the hydrophobic driving force for binding neutral membranes. Indeed, preliminary data suggest that selectivity is significantly higher at [*killer*FLIP-E]=1 μM than at 0.1 μM. These results indicate control of aggregation as a new principle in the design of selective anticancer peptides.
[1] B. Pennarun *et al.* Cell Death Dis 2013 4 e894
[2] G. Bocchinfuso *et al.* J Pept Sci 2009 15 550

Abstracts
– 16. Membranes and Vesicles –

L-897

Cholesterol stiffens unsaturated lipid bilayer via pairing with lyso-lipids

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Membranes of eukaryotic cells contain especially high amounts of cholesterol so that it becomes a critical determinant of structural, thermodynamic and elastic properties of the membrane. The bulk effects of cholesterol are generally associated with its interaction with fatty acid chains of membrane lipids. Cholesterol induces pronounced ordering in fluid lipid bilayers containing saturated fatty acids. This ordering is associated with the dramatic increase of membrane stiffness. Less studied is the effect of cholesterol on unsaturated lipid bilayers, where even high (30mol%) amounts of cholesterol produce negligible changes in membrane rigidity. Nevertheless, we show here that the bending rigidity of unsaturated lipid bilayers can be enhanced up to 5-fold by adding cholesterol in equimolar concentration with lyso-lipids. We found that cholesterol inhibited pore-forming activity of lyso-lipids. It also severely diminished the curvature-driven lateral redistribution of lyso-lipids. We further associated these observations with ability of lyso-lipids and cholesterol to form metastable bimolacular complexes via hydrogen bounding. We discuss the possible mechanisms of this effect related to orientation of cholesterol in the lipid bilayer.

L-898

The role of SPB-chromosome attachments for nuclear envelope shape in fission yeast mitosis

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During closed mitosis in fission yeast *Schizosaccharomyces pombe*, the nuclear envelope experiences a process of symmetrical shape transformations when the spindle pole bodies (SPBs) are pushed apart by elongating spindle microtubules. Here we show that the symmetry of the dividing fission yeast nucleus is assured by SPB-chromosome attachments, which can be ascribed to the formation of kinetochore clustering in the vicinity of SPBs. These transformations do not correspond to the behavior of unsupported lipid vesicles whose shapes can be determined by the minimization of the membrane bending energy. We developed a simple model that explains the symmetrical dumbbell shapes of the nuclear envelope on the basis of forces exerted on the membrane by chromosomes clustered at SPBs. In this way we determine the formation of otherwise energetically unfavorable dumbbell structures upon spindle elongation, assuming that fission yeast nuclear envelope exhibits the same mechanical properties as lipid vesicle membrane. The model predicts the appearance of abnormal asymmetric shapes in fission yeast mutants with missegregated chromosomes as well as altered nuclear membrane composition.

L-899

Horizontal lipid nanotubes as substrates for membrane scission, probed using an opto-electric setup

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Understanding the action of membrane scission machines requires physiologically-relevant membrane substrate analogues. Such analogues are currently available in the form of membrane nanotubes drawn from giant unilamellar vesicles (GUVs); however, accessibility of the vesicle lumen is restricted, thereby limiting control over internal solute composition. Notably, this prevents investigation of luminally-binding proteins, for example, the ESCRT III membrane scission complex. Resolution of such issues can be achieved by drawing nanotubes from vertically-inclined black lipid membranes, which can be coupled with microfluidic sample delivery systems to permit environmental control at both membrane interfaces, mimicking changes in the cytosol. These substrate-analogues provide improved membrane access, whilst adding compatibility with fluorescence microscopy and patch-clamp techniques, yielding sub-micron spatial and sub-second temporal resolutions. This unlocks the ability to investigate the mechanism the ESCRT-III complex in a unique manner: Through simultaneous measurement of the coordination between fluorescence increase upon binding of the polymeric CHMP subunits and the associated reduction in conductance arising from nanotube constriction.

L-900

Cell Membrane-derived polymer-supported lipid bilayers with preserved transmembrane protein mobility

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A generic protocol for producing polymer-supported lipid bilayers that contain native membrane components which retain transmembrane protein mobility and activity will be discussed. By merging vesicles containing synthetic lipids (PEGylated lipids and POPC lipids) with native cell membrane vesicles (a.k.a., plasma membrane preparations or ghost cells) hybrid vesicles where generated which readily rupture on glass. The role of the PEG-cushion during SLB formation and its effect on transmembrane protein lateral mobility will be discussed. Specific activity studies indicate that transmembrane protein targets were unadulterated by the vesicle preparation process and function was also observed in the hybrid SLB. Thus, this strategy for the facile production of SLBs from native cell membranes should prove to be a valuable tool for generating biomimetic surfaces for use in studying both pathogen-host and cell-cell interactions.

Abstracts

– 16. Membranes and Vesicles –

L-901

Electrofusion of Cell-GUV enables micrometer-sized artificial objects transfer into live cells

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Introduction of artificial objects into live cells is an important topic in biotechnology. Several powerful methods have been used for the purpose such as lipofection, virus vector, and electroporation. However, these methods are limited to treat small sized objects in the range of tens nanometer. We have developed the novel method for introducing artificial objects ranging in size from 10nm to 1μm into live cells, by using electrofusion with an artificial giant unilamellar vesicle (GUV, liposome) [1]. Briefly, dispersed cells and GUVs were placed into electrofusion chamber, and were exposed to an AC field to align cells-GUVs, and were also exposed DC pulse to induce transient electrofusion. The processed cell reached confluence with entrapping specified sizes of fluorescent beads. The transfer efficiency was evaluated by using fluorescence microscopy and flow cytometry. Delivering magnetic beads, DNA origamis, plasmids were also implemented. In this talk, we will describe the novel method and effective parameters for the transfer efficiency. We believe that the method will be used for elucidation of cell mechanisms and even creation of artificial cells.

This work was supported by JSPS grants (#15H02774, #22220001, #24104004, #25610117)

[1] Saito A. C. *et al.*, PLOS ONE, **9**, 2014.

L-902

Phosphatidylcholine liposomes as ultra efficient boundary lubricants

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Lipids are likely associated with physiological lubrication, however the importance of lipid structure and composition for efficient lubrication remained, until now, unclear. We have explored the origins of boundary lubrication by phosphatidylcholines. First, a systematic study of normal and shear forces between two opposing surfaces bearing PC vesicles/bilayers was performed. We have studied the lubricating properties of different liposomes as a function of their acyl chain length, and demonstrated that overall liposome lubrication ability improves markedly with increasing length of the acyl chain, and correlates strongly with the liposomes' structural integrity on the substrate surface. A further comparison between DSPC SUVs and DSPC bilayers showed reduced mechanical strength of bilayers compared to liposomes, as revealed by an AFM force spectroscopy study. In correlation with this, less efficient lubrication of bilayers was observed in SFB measurements. Deeper understanding of lubrication by liposomes and bilayers sheds light on the lubrication mechanism in synovial joints, and consequently, will help to design better scaffolds and implants for treatment of joint diseases.

Abstracts
– 18. Protein Folding, Assembly and Stability –

L-903

The Influence of Physical Interactions on Kinetics, Structure and Dynamics of Amyloid β Peptides

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The capability of proteins of building a well-defined misfolded structure, so called amyloid fibrils, seems so be a generic property of proteins. Interestingly, although proteins are very different in their native conformation, the amyloid fibrils share a common structural motif. The so called cross-β structure consists of two β-sheets and is characterized by two typical distances: 4,7 Å between β-strands and 9-11 Å between the two sheets. In this work, physical constraints are introduced in a very well described system: Aβ(1-40). The strong hydrophobic contact between F19 L34 is altered by charges, hydrophobicity, electrostatic repulsion and attraction. The influences of this mutations are studied concerning kinetics, structure and dynamics of the system. Used methods are electron microscopy, x-ray diffraction, fluorescence spectroscopy and solid state NMR spectroscopy. Although drastic local changes occurred in some cases, the overall fibril structure remained the same and seems therefore very robust.

L-904

A sensor for quantification of macromolecular crowding in living cells

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The cell is highly crowded with biomacromolecules, and the excluded volume influences processes such as diffusion, folding, conformation, and aggregation or association of proteins and polynucleic acids. In *Escherichia coli*, the values reported for the total macromolecular content range from 200 to 400 mg/mL. Knowledge of the macromolecular crowding is needed to understand behavior and especially interactions of biomolecules in vivo, be it for drug development, fundamental knowledge, or to support computational efforts to model the living cell. Direct spatiotemporal read-out of the crowding would be a powerful asset to unravel the structure of the cytoplasm and the impact of excluded volume on protein function in living cells. Here, we introduce a Förster resonance energy transfer (FRET) sensor for quantification of the macromolecular crowding and apply the sensor in living cells.

L-905

The protein folding problem: bottom-up or up-down?

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We hypothesize that few non-local interactions are effective in the early phases of the folding transition prior to the cooperative transition. These interactions loosely stabilize few closed loops which form the folding non-contiguous nucleus, reduce the chain entropy and determine the course of the folding pathway (the "loop hypothesis"). We study the order of formation of secondary structure elements and long loops' closure transitions during the early phases of the folding of *E. coli* adenylate kinase (AK) by combination of rapid mixing methods and time resolved FRET spectroscopy. We find that at the initiation of folding of the AK molecule the two N terminal closed loop structures in the CORE domain reach native end to end distance within the first 60 microseconds of the transition. Three representative CORE domain β-strands have non-native end to end distance during the first 15 ms and undergo slow change (3 sec) to native distance. Not all helical segments in the CORE domain fold at the slow rate as the beta strands. We conclude that non local interactions are essential factor at the early phases of the folding transition and the loop closure transition forms a nucleus that serves as a "mold" for the folding of the rest of the chain.

L-906

Convertible assembly forms of human lamin A: filaments and paracrystals

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Lamins are intermediate filament proteins forming a meshwork of fibers at the inner nuclear membrane of metazoan nuclei. Lamins harbor an extended alpha-helical rod flanked by non-alpha-helical "head" and "tail" domains. Starting from coiled-coil dimers, we investigated the assembly mechanism of human lamin A with regard to the contribution of these individual sub-domains on assembly by analytical ultracentrifugation and electron microscopy. Moreover, we explored the impact of spatial confinement on lamin filament organization. By restricting lamin filaments to a thin film of buffer on a charged surface, they promptly convert from a filamentous to a paracrystalline order. Moreover, filaments assembled under physiological conditions convert into paracrystalline fibers within seconds when the ionic strength is lowered indicating that extended filament systems are able to dynamically reorganize into paracrystals. Moreover, the formation of filaments is bypassed in favor of 3-dimensional lattices of paracrystalline fibers, when lamin A dimers are dialyzed from high-salt into physiological buffers. We suggest that the potential for a regulated interconversion of lamin A filaments and paracrystalline arrays may be important for nuclear organization in certain physiological situations.

Abstracts

– 18. Protein Folding, Assembly and Stability –

L-907

Single-molecule FRET in fused silica capillaries under high pressure

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Single-molecule fluorescence experiments are typically performed in chambers made of microscope cover slips. While these chambers are relatively straightforward to make, they have several disadvantages: The sample volume is still pretty large, application of flow requires special channel structures, and particularly the application of high pressures is not possible. Fused silica capillaries on the other hand may be a relatively straightforward to implement alternative enabling experiments under flow or high pressures.
We show experiments in a square bore fused silica capillary with an inner diameter of 50 μm and an outer diameter of 300 μm. The dimensions of the capillary are perfect for the use on a microscope with a wall thickness compatible with high NA water immersion microscope objectives. Square Capillaries can stand pressures up to 2000 Bar. We characterize the influence of beam distortions by fluorescence correlation spectroscopy (FCS) and photon-counting histogram (PCH) analysis and improved the optical properties of the capillary by placing the capillary on a 100 μm thin silica coverslip.
With chemical denaturants and pressure we aim at unfolding the Cold shock Protein A. We performed single-molecule FRET-measurement in the capillary on the donor-acceptor-labeled Protein with increasing pressure up to 2000 Bar and compared it with chemical denaturation via GdnHCL.

L-908

Aggregation mechanism of Aβ42 familial mutants resolved over microscopic steps

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Several lines of evidence link aggregation of amyloid-β peptide (Aβ) to the pathogenesis of Alzheimer's disease (AD). Aβ is cleaved from amyloid precursor protein; Aβ42 is a main variant. Familial mutations, A21G (Flemish), E22K (Italian), E22G (Arctic), E22Q (Dutch) and D23N (Iowa) are linked to early-onset AD. We measure ThT fluorescence as a function of time and use global fitting to address the dominant aggregation pathway. All five mutants show sigmoidal curves in a concentration dependent manner and reduced aggregation half time compared to Aβ42 wild type (wt) at physiological concentrations. E22G is the most aggregation prone variant, followed by D23N, E22Q, and E22K, whereas A21G behaves more close to Aβ42 wt. From kinetic analyses, a two-step saturated secondary nucleation dominating aggregation process is suggested for all five mutants, which means that secondary nucleation is dominant and monomer dependent at low monomer concentration and monomer independent at high monomer concentration. Seeding experiments confirm the importance of secondary nucleation. This mechanistic change could be mainly attributed to reduced electrostatic repulsion, except for A21G.

Abstracts
– 19. Channels and Transporters –

L-909

Smith-Lemli-Opitz syndrome modifies gating of Kv1.3 channels of T lymphocytes

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The Smith-Lemli-Opitz syndrome (SLO) is a multiple congenital anomaly, caused by a decreased or abolished activity of 7-dehydrocholesterol (7DHC) reductase. Increased 7DHC/cholesterol in SLO ratio may modify the physico-chemical properties of plasma membrane, and hence may influence the operation of the ion channels in many cell types including T cells.To test this hypothesis we compared the biophysical properties of Kv1.3 channels in T cellsof SLO patients (SLO-T-cells), T-cells of healthy volunteers loaded with 7DHC and control T cells. T lymphocytes were isolated from the peripheral blood of volunteers and patients with SLO. 7DHC elevation in T lymphocytes membrane was achieved upon treatment with cyclodextrin/7DHC complex. Our results showed that both activation and inactivation kinetics were significantly slower, and the midpoint of the steady-state act. was shifted toward positive voltages in SLO. Qualitatively and quantitatively differences in the gating of Kv1.3 channels were observed in 7DHC-T-cells vs. control.T-cells from SLO patients had decreased proliferation rate as compared to healthy controls. These data demonstrate that elevated 7DHC level of cell membrane can modify the operation of ion channels and may contribute to the neurodegenerative defects in SLO.

L-910

Modulation of glutamate receptor ion channels by plasma membrane cholesterol

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The most common synapses in human brain are those where glutamate is released as neurotransmitter. NMDA and AMPA/kainate receptors are ion channels residing in postsynaptic membrane of these synapses and their ion channels open upon binding glutamate. We examined how cholesterol, which is a natural component of plasma membrane, affects NMDA and AMPA/kainate receptors.
Cholesterol depletion of cultured rat cerebellar granule cells was done by supplementing the medium with methyl-beta-cyclodextrin. We used patch clamp technique to directly observe the function of glutamate receptor ion channels. The receptors were activated by fast application of solutions containing specific agonists: NMDA for NMDA receptors and kainate for AMPA/kainate receptors.
Cholesterol depletion results in robust (97%) decrease of NMDA receptor function. This is underlain by sharp decrease of ion channel open probability. Moreover, cholesterol depletion results in stronger and faster desensitization of NMDA receptors. Surprisingly, AMPA/kainate receptor function is independent of cholesterol content.
Our data show that plasma membrane cholesterol is necessary for NMDA receptor ion channel opening while cholesterol does not affect AMPA/kainate ion channel.
Supported by GACR P303/12/1464; P304/12/G069.

L-911

Cloning and tissue specific expression pattern of Astacus leptodactylus Sodium/Calcium Exchanger

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Cytosolic Ca^{2+} plays a fundamental role in various cellular functions. Sodium calcium exchanger (NCX) is an essential component in cellular Ca^{2+} regulation. Exchanger is present among almost in all form of organisms, from unicellular creatures to higher animals. Functional similarity may indicate a possible conserved homology pattern in molecular structure of the exchangers within the species. Recently we have cloned an open reading frame for NCX gene in the crayfish (*Astacus leptodactylus*). Comparison of the sequence of the novel α peptide indicated a 60 % of homology to the other known NCX sequences. Molecular topology analysis of the amino acid sequence revealed a signal sequence at N-terminal, two highly hydrophobic domains (consisted of 10 transmembrane segments) at each ends of the peptide which are flanking a large intracellular loop responsible for ion binding and functional regulation. A higher homology rate was observed for transmembrane domains (up to 76%) as compared to that for intracellular loop (up to 60%). Future works, would be dedicated to heterologous expression and functional study of the novel NCX gene. This study was supported by TÜBITAK (grant # 113S555, BIDEB 2210) and Hacettepe University (grant #014D08101006 and #013D03101003).

L-912

The N-terminal domain acts as a mechanosensor for gating of the E-coli 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 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. However, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. Thus we performed MD simulations for the opening of MscL. Upon membrane stretch, Phe78 was dragged by lipids with stronger interactions with lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. Neighboring TM1s cross and interact with each other near the cytoplasmic side through hydrophobic interaction between Leu19-Val23 in one TM1 and Gly22 in the neighboring TM1, forming the most constricted hydrophobic part of the pore called gate. Upon membrane stretch, the helices are dragged and tilted, accompanied by the outward sliding of the crossings, leading to expanding of the gate. In this study, we assessed how important of the N-terminal (S1) helices running parallel to the cytoplasmic membrane for sensing membrane tension. As a result, the some amino acids in S1 sense membrane tension as much as Phe78.

Abstracts

– 19. Channels and Transporters –

L-913

Mechanosensitive ion channels in differentiated and de-differentiated chondrocytes

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Chondrocytes alter their production of extracellular matrix molecules in response to changing mechanical loads. For many years mechanically-gated ion channels have been implicated in this process, however direct mechanical gating of channels in these cells had not been demonstrated. We used elastomeric pillar arrays to apply the mechanical stimuli while monitoring membrane currents using whole-cell patch-clamp. We found that chondrocytes, when stimulated at the cell-substrate interface exhibit robust, transient, inward currents. As these cells de-differentiate *in vitro*, these currents become larger and more sensitive. The currents in chondrocytes were reversibly blocked by the application of GSK205, a TRPV4-specific antagonist. To confirm that TRPV4 can mediate such, we over-expressed TRPV4 in HEK cells and found deflection-gated currents which were reversible blocked by the antagonist. In contrast, when the over-expressed TRPV4 in HEK cells were subjected to pressure steps in pressure-clamp inside-out configuration we barely detect a stretch-sensitive response. These data suggest that the coupling of TRPV4 into structures at the cell-substrate interface leads to channel gating not observed when stimuli are applied by only stretching the exposed surface of the cell.

L-914

Exchange of sodium or potassium ions with protons on cytoplasmic side of Na,K-ATPase

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Electrogenic exchange of ions through the access channel to the ion-binding sites on cytoplasmic side of the Na,K-ATPase has been studied and analyzed. Small capacitance changes were measured using combined membranes consisting of the membrane fragments densely packed with Na,K-ATPase attached to a bilayer lipid membrane (BLM) or to a solid supported membrane. The capacitance changes were triggered by a pH jump initiated by proton release from a photosensitive compound (caged H^+) and depended on the initial pH and the concentration of sodium or potassium ions. The effects of these ions are explained by competition of protons with sodium or potassium ions in the binding sites accessible from the cytoplasmic side of the Na,K-ATPase. The approximation of the experimental data by theory-derived curves yields the dissociation constants and the cooperativity coefficients of the binding sites for sodium and potassium ions. These parameters depended on the presence of magnesium ions and ATP. The latter effects may be explained as an electrostatic or conformational effect of magnesium ions bound to a separate site close to the entrance of the access channel of the Na,K-ATPase and a shift of the conformational equilibrium of the Na,K-ATPase by ATP bound to the low-affinity site.

Abstracts
– 20. Cell Biophysics and Signaling –

L-915

Antiproliferative effect of doxorubicin/quercetin/menadione combination in leukemia Jurkat T cells

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The antiproliferative effect of the anticancer drug doxorubicin (DOX) combined with the flavonoid quercetin (QC) and menadione (MD) was investigated in human leukemia Jurkat cells. Cell cycle, apoptosis/necrosis and oxidative status were assessed by flow cytometry. In 18-h treatments, DOX dose-dependently decreased the viable cell fraction (IC_{50} = 0.57 μM). The 15 μM equimolar QC/MD combination produced by itself 52% cell death rate, associated with oxidative stress generation and apoptosis induction, and enhanced DOX cytotoxicity, producing a dramatic decrease in the viable cell fraction (IC_{50} = 1.25 μM). 15 μM QC combined with 7.5 μM MD produced 69% viable cells, and association with DOX exhibited additive cytotoxicity (IC_{50} = 2.26 μM). 0.1 μM and 1 μM DOX selectively arrested the cell cycle in G_2/M (63% cell fraction) and S phase (70%), respectively. Addition of QC/MD up to 7.5 μM and 2.5 μM equimolar ratio, respectively, further increased the S-cell fraction. Higher levels progressively increased the G_0/G_1 cell fraction. In conclusion, the QC/MD combination could improve the therapeutic index of doxorubicin. Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0800.

L-916

Periostin promotes epithelial mesenchymal transition via p38/ERK/miR-381 pathway in lung cancer

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Periostin is a multifunctional cytokine that signals between the cell and the ECM. Recently studies have been recognized, initially in epithelial cancer, that mature epithelial cells can undergo a second round of EMT, leading to invasive, motile cell type. The functions and molecular mechanisms of miRNAs in regulating EMT in cancer metastasis have been discussed. Our result showed that treatment with periostin promoted EMT in lung cancer A549 cells. Moreover, EMT marker Twist expression was associated with EMT phenotype in different lung epithelial cell. We also found that periostin may regulate EMT through p38/ERK pathway in A549 cells. Moreover, periostin also regulate EMT through miR-381 post-transcriptional regulation.We had been also establish the periostin knockdown stable cell line, and the data showed that periostin knockdown affected EMT markers expression and decreased migration potential in vitro. Finally, our IHC results showed that high expression levels of periostin were investigated in lung cancer specimens. This study will demonstrate whether periostin promotes EMT in lung cancer, and may provide the opportunity to develop a novel therapeutic target for lung cancer progression.

L-917

The kinetics of ligand – receptor binding at cell surfaces: multivalency and binding mode

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TRAIL (Tumor Necrosis Factor Receptor Apoptosis Inducing Ligand) appears as an interesting candidate for cancer therapy as it induces apoptosis in cancer cells without toxicity to normal cells. Understanding the relation between TRAIL binding mode and apoptosis would support rational drug development. TRAIL is engaged in multivalent interactions with various receptors, making the monitoring and quantitative description of binding challenging. We have applied innovative technologies to investigate the binding kinetics of synthetic monovalent and bivalent TRAIL mimics to the receptors. Their binding to TRAIL receptors at the surface of living cancer cells was monitored with Ligand Tracer®(Ridgeview Instruments). Their binding to recombinant TRAIL-R2 at sensor surfaces was measured with Biacore®(GE-Healthcare Biacore). The kinetic curves were evaluated by kinetic distribution analysis (InteractionMap®, Ridgeview Diagnostics). The analysis yields two-dimensional maps (association versus dissociation rate constant) where each peak represents the affinity of one of the components that contribute to the binding curve. The binding data will be discussed in relation with the apoptotic activity of the peptides.

L-918

CCN3 induces epithelial mesenchymal transition by FAK/Akt/HIF-1α pathway in prostate cancer

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Prostate cancer (PCa) is the most commonly diagnosed malignancy in men in the United States and other Western countries. Epithelial to mesenchymal transition (EMT) has received considerable attention as a conceptual paradigm to explain metastatic behavior during cancer progression. CCN3, a matrix-associated protein involved in many cellular functions. A previous study showed that CCN3 expression is upregulated in PCa cells and human PCa patients. However, the roles of CCN3 in EMT regulation in PCa are poorly understood. Our data showed that CCN3 expression level is associated mesenchymal phenotype in PCa cell lines. Moreover, treatment with CCN3 promotes EMT in PCa cells. We have been also establish the CCN3 overexpression and knockdown stable cell lines and the results showed that CCN3 overexpression promoted EMT but this effect is inhibited in knockdown cells. We also found that CCN3 may promote EMT by activating FAK/Akt/HIF-1α pathway in PCa. Finally, the IHC staining showed that expression level of Twist was positively correlated tumor stage and bone metastasis in prostate specimens. The present study will delineate whether CCN3 modifies EMT regulation of the prostate cancer, and may provide the opportunity to develop a novel therapeutic target for PCa progression.

Abstracts

– 20. Cell Biophysics and Signaling –

L-919

Growth-suppressive action of doxorubicin on human leukemia jurkat cells. Modulation by quercetin

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The cytotoxicity of the anticancer drug doxorubicin (DOX) in human leukemia Jurkat T cells was assessed by flow cytometric determinations of cell cycle, apoptosis/necrosis and oxidative status. 18-h DOX-exposure induced apoptosis with IC_{50} = 951 nM, which was accompanied by significant oxidative stress generation (IC_{50} = 620 nM). The sub-G_0 cell fraction consistently increased in a highly cooperative manner (IC_{50} = 434 nM). DOX induced cell cycle arrest displaying a trimodal distribution, so that low, moderate and high doses of DOX preferentially produced G_2/M, S and G_0/G_1 blockage with IC_{50} of 49 nM, 464 nM and 1866 nM, respectively. The flavonoid quercetin (QC) (15 μM) exerted strong antioxidant effects, reducing DOX-induced oxidative stress and early cell death (IC_{50} = 2119 nM and 4897 nM, repectively). However, cell cycle arrest induced by low and moderate doses of DOX was maintained in the presence of QC levels <25 μM. JC-1 fluorimetric assay revealed that DOX induced substantial mitochondrial hyperpolarization within 4 h, which was potently reversed, up to complete depolarization, by QC (IC_{50} = 1.2 μM). Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0800.

L-920

Characterization of a prototypical mechanochemical pattern generator in PAR polarity of *C. elegans*

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Classical biological patterning mechanisms like morphogen gradients of Turing patterns rely on diffusion of morphogens. The impact of forces and flows on morphogenetic pattern formation are largely unexplored, despite its well-recognized importance. Here we uncover a new class of a pattern-generating motif that is founded upon feedback between mechanical forces and biochemical regulation, in the emergence of partitioning-defective (PAR) polarity of the *C. elegans* zygote. By linking quantitative concentration and flow measurements to a physical theory, we demonstrate that mechanochemical feedback amplifies cortical flows and promotes a rapid transition to the patterned state of the PAR system. Additionally, we found evidence that flow amplification is essential for the robustness of the mechanochemical patterning motif. We anticipate that this characterization of a mechanochemical patterning motif will open new avenues in quantitatively understanding the emergence of patterns during the development of an organism.

L-921

Chemotherapeutic potential of the doxorubicin/menadione combination in a human leukemia cell model

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We show that menadione (MD) can potentiate the cytotoxicity of the anticancer drug doxorubicin (DOX) in human leukemia Jurkat T cells. Cell cycle, apoptosis/necrosis and oxidative status were assessed by flow cytometry on propidium iodide, Annexin V-FITC/7-AAD and CM-H_2DCFDA/7-AAD labeled cells, respectively. Within 18 h of exposure, DOX induced oxidative stress and decreased the viable cell fraction in a dose-dependent and cooperative manner (IC_{50} = 0.6 μM, Hill coefficient H = 2). 15 μM MD enhanced consistently oxidative stress generation and increased the cooperativity of DOX (H = 4). 7.5 μM MD also generated significant oxidative stress but did not affect DOX cooperativity. Both DOX and MD were potent apoptogens in Jurkat cells. A low level of 0.1 μM DOX efficiently arrested the cell cycle in G_2/M. MD apparently abrogated the DOX-induced cell cycle blockage by promoting growth arrest and apoptosis in a highly cooperative process (IC_{50} = 14 μM, H = 5). JC-1 fluorimetric assay indicated that MD reversed, with an IC_{50} of 4.8 μM, the DOX-induced mitochondrial hyperpolarization observed after 4 h from exposure. Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0800.

L-922

Feedback mechanism for microtubule length regulation by bistable stathmin gradients

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Microtubule (MT) polymerization dynamics is regulated by the signaling proteins Rac1 and stathmin. In cells, the MT growth rate is inhibited by the soluble protein stathmin, which, in turn, is inactivated by Rac1. Growing MTs activate Rac1 at the cell edge, which closes a positive feedback loop. Assuming that Rac1 is activated by MT contact at the cell edge, we formulate and analyze a model for MT growth regulated by Rac1 localized at the cell edge and cytosolic stathmin.

For a homogeneous stathmin concentration in the absence of Rac1, we find a switch-like regulation of the MT mean length by stathmin. For constitutively active Rac1 at the cell edge, a spatial gradient of active stathmin is established. In this gradient, we find a stationary bimodal MT length distributions. One sub-population of the bimodal length distribution can be identified with "pioneering" MTs in the region near the cell edge, which have been observed experimentally. Rac1 activation by MTs closes the feedback and establishes a bistable switch with two stable states: one stable state corresponds to upregulated MT growth with "pioneering" MTs; the other stable state corresponds to an interrupted feedback with short MTs. Stochastic effects as well as external perturbations can trigger switching events.

Abstracts
– 20. Cell Biophysics and Signaling –

L-923

How to transform a toroid into a spheroid: self organization in tissue regeneration and morphogenesis

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Tissue cells may exist as individuals; but in first line their destination is to contribute to self-organization in the tissue context. Our question is how those tiny cells can shape large complex structures as the lung, heart or other organs. In 2003 C.F. proposed the mechanical stress field as a fast, robust and efficient communication pathway permitting morphogenesis and tissue repair (C. Fütterer, C. Colombo, F. Jülicher, A. Ott, Europhys. Lett. 64, 137, 2003). In order to study this question we selected Hydra vulgaris as a model organism because of its structural simplicity and robustness. In addition it performs a genuine symmetry breaking scenario as it always passes through a spherical state during regeneration. Therefore it seems to us the ideal system for precise measurements and mathematical modelling. We present our experimental and theoretical results with regenerating tissue toroids revealing a pitchfork bifurcation to an extremely fast folding process triggered by a super-cellular actin ring. Furthermore we ask what happens when mechanically stimulating these toroids during regeneration. Our experimental results performed with our new patented tissue stretching device show intriguing contractile dynamics not understood so far. We would like to motivate the superiority of toroids to spheroids as a universal geometry for tissue studies because of many advantages.

L-924

Analysis of metrics for molecular sonotransfer *in vitro*

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Ultrasound induced microbubble (MB) cavitation is widely used to increase delivery of drugs and genes into the cells and tissues. In present study, we have simultaneously performed quantitative evaluation of three main sonoporation factors: 1) MB concentration, 2) MB cavitation extent and 3) doxorubicin (DOX) sonotransfer into Chinese hamster ovary cells. MB concentration measurement results and passively recorded MB cavitation signals were used for MB sonodestruction rate and spectral root mean square (RMS) calculations, respectively. Subsequently time to maximum value of RMS and inertial cavitation dose (ICD) quantifications were performed for every acoustic pressure value. This comprehensive research has led not only to explanation of relation of ICD and MB sonodestruction rate, but also to the development of new sonoporation metric: the inverse of time to maximum value of RMS (1/ time to maximum value of RMS). ICD and MB sonodestruction rate intercorrelation and correlation with DOX sonotransfer suggest inertial cavitation to be the key mechanism for cell sonoporation. All these metrics were successfully used for doxorubicin sonotransfer prediction and therefore shows feasibility to be applied for future dosimetric applications for ultrasound-mediated drug and gene delivery.

L-925

Quantifying membrane permeability during the formation of amphotericin B ion channels in single living cells

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Recently, the structure-function relationships between amphotericin B (AmB) and ergosterol has been solved using synthetic technique. We investigate the fundamental question about whether there exists the concentration- and time-dependence of AmB induced permeability changes across ergosterol-containing membrane. Present results show that the pore size of the AmB ion channels is in an AmB dose-dependent manner; this consequence has been proved by two fluorescent dyes of known average diameter. In addition, AmB ion channel formation is not causally linked to cytotoxicity in HT29 cells when the pore size of the AmB ion channels is falling in the range of 8 Å- 17.56 nm, where the pore size of single AmB ion channel is 8 Å. On the other hand, based on our intracellular microrheology assay, the results reveal an increase in both velocity fluctuations and diffusion coefficients of vesicles within the living HT29 cells due to the presence of AmB ion channels.

L-926

Force generation of blood platelets – with and without physiological flow conditions

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Human blood platelets play an essential role in early wound closure. They attach to the wounded tissue, spread on the extracellular matrix and contract to form a blood clot as a temporary seal. *In vivo*, they circulate in the blood vessels and are thus naturally exposed to flow. We study the contraction of platelets in a stationary environment using time-resolved Traction Force Microscopy (TFM). We seed the cells on polyacrylamide gels containing fluorescent beads and calculate the contractile forces using a PIV-algorithm. Furthermore, we established a tool to study the contraction of blood platelets in a flow environment, mimicking their natural surroundings in blood vessels. A flow chamber is combined with the existing TFM experimental setup. The flow rates can be adjusted to imitate the shear rates of venous or arterial blood flow of 100 s^{-1} or 1000 s^{-1}, respectively. This combination of tools will enable us to study the contractile forces of blood platelets and compare the temporal evolution of the force fields for stationary and flow conditions. Additionally, the flow chamber provides the possibility to add certain drugs or reagents at specific time points to study their influence on the contractility of the platelets.

Abstracts

– 20. Cell Biophysics and Signaling –

L-927

A co-culture platform for unculturable, marine bacteria

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A co-culture chip for marine cells has been designed and fabricated. The chip design was based on the MicroDish Culture Chip (MDCC) and comprised an alumina membrane support (AOM) with photopatterned SU8-2050 chambers subdivided by a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel wall. The chip design enables different cell types to talk to each other with chemical messenger molecules while maintaining spatial separation of the cells. This communication is thought to be essential for the culturability of a range of marine microorganisms.
Extensive tests of the chip showed that the pores in the AOM were not blocked and remained accessible for growth medium. Swelling and diffusion of small molecules through pHEMA depended on cross linker concentration. However, even for the lowest crosslinker concentration of 0.1% mol/mol swelling of pHEMA remained restricted to a maximum of $\sim 20\%$, and the diffusion coefficient of methylene blue was found to be $6 \times 10^{-8} cm^2/s$ pHEMA i.e. close to that of pure water. Initial tests with bacterial cells (*B. subtilis*) and yeast (*S. cerevisiae* and *Y. lipolytica*) showed that the chip was suitable for coculture. Future experiments will be aimed at demonstrating successful cell-cell interaction and the improvement of the culturability of marine bacteria.

L-928

Mechanical coupling between the cytoskeleton and the nucleus

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It is nowadays widely acknowledged that mechanical cues are as important for cellular behavior as traditional biochemical ones. Strikingly, adult stem cells can be guided to differentiate towards various cell types when cultured on elastic hydrogels with appropriate Young's modulus E. While the differentiation process takes several days, the acto-myosin cytoskeleton organization shows significant differences within the first 24 hours after plating. We investigate the mechanical properties of the nucleus by atomic force microscopy and fluorescence microscopy and demonstrate the impact of substrate elasticity E on nuclear morphology via acto-myosin stress fibers. Elucidating the mechanical coupling of the cytoskeleton and the nucleus might reveal a direct mechanical pathway that alters gene transcription and might impact adult stem cell differentiation.

L-929

Interactions of nanoparticles with cells in culture

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The fate of lipid coated gold nanoparticles internalized in adherent cells in culture is studied using fluorescence and confocal microscopy imaging of the gold nanoparticles and markers for cellular internal compartments involved in the endocytic process. Using Image Correlation Spectroscopy, Image Cross-Correlation Spectroscopy, and Image Triple Cross-Correlation Spectroscopy, it is possible to determine the fraction of nanoparticles associated with each intracellular compartment and the fraction of the compartments involved in the internalization process. This provides insight into the pathway of internalization and the processing of the nanoparticles by the cells. It appears that the pathway depends on the type of cell studied.

L-930

Probing the effect of EGFR tyrosine kinase inhibitor (PD153035) on EGF-induced chemotaxis by a single-cell approach

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Recently, several approaches have been devoted to the study of the chemotactic response of cancer cells, including transwell assay, wound-healing assay, micro pipette, microfluidics, and microparticles. However, when the chemoattractant is expensive or limited in quantity, a reduction in chemoattractants and precise control microenvironments of cells are needed. Herein, we present an optical tweezers-based approach to regulate the directional locomotion during epidermal growth factor (EGF) chemotaxis. Our idea is that using streptavidin-coated bead conjugation to EGF as a point source of a chemoattractant to locally stimulate HT29 cells. We then apply optical tweezers system to conduct spatial and temporal regulation of cell locomotion, where optically trapped bead is coated with the chemoattractant EGF. We have demonstrated that the chemosensing is directly mediated by epidermal growth factor receptor (EGFR) signaling. In addition, the locomotion of HT29 cells depended on whether the cells sensed the presence of the chemoattractant EGF. Stimulation of HT29 cells with EGF-coated beads induced locomotion, but locomotion was not stimulated by streptavidin-coated beads without EGF or by EGF-coated beads in the presence of the EGFR tyrosine kinase inhibitor PD153035.

Abstracts

– 20. Cell Biophysics and Signaling –

L-931

Elastic behavior of HBL-100 cells on soft and hard substrates probed by optical tweezers

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Cancer has multifaceted nature, starting from its initiation to progression and finally metastasis. The quest for different biophysical hallmarks is continued to understand the mechanism which helps in diagnosis and cure. One of the traits is elasticity of the cells and its dependence on microenvironment. Elasticity, a potential label free indicator for cellular alteration, has been widely investigated for cancer and similar diseases. We used optical tweezers (OT) to study the elasticity of HBL-100 cells at low forces (<10pN) on bare and collagen coated substrates. Elastic modulus measured during cell indentation was 26±9 Pa for the bare and 19±7 Pa for collagen-coated substrate. A similar trend is observed during the retraction of the cell: 23±10 Pa and 13±7 Pa, respectively. These results show the cells adapt their stiffness to that of the substrate and demonstrate the potential of OT for low-force probing for modifications to cell mechanics induced by the surrounding environment.

Abstracts
– 22. Protein-Lipid Interactions –

L-932

Neutron reflection reveals initial steps in pore formation by a eukaryotic cytolysin

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Equinatoxin II (EqtII) is a pore-forming cytolysin from the venom of sea anemones. It efficiently lyses cellular membranes by a mechanism involving a conformational change that, following initial binding, insert the N-terminal α-helix into the membrane. In this study we have used neutron reflection to determine the structures of EqtII-membrane assemblies *in situ*. We discovered that EqtII has several different modes of binding to membranes depending on the lipid composition. EqtII interacts weakly and reversibly with pure dimyristoyl-phosphatidylcholine membranes, with an orientation approximately parallel to the membrane surface. The presence of sphingomyelin gives rise to a more upright orientation of EqtII, but cholesterol is needed for deeper insertion into the membrane. Cooling the EqtII-lipid assembly below the lipid phase transition temperature leads to water penetration deeper in the membrane and a significant reduction in the extension of the protein outside the membrane. These results help to clarify the early steps in pore formation by EqtII and highlight the valuable information on protein-membrane interactions available from neutron reflection measurements.

L-933

Selective lipid co-aggregation with amyloid fibrils

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In amyloid plaques, associated with several amyloidogenic diseases, tightly associated lipids have been identified. For several of the amyloid disorders, protein aggregation has also been associated with membrane disruption. Parkinson's disease (PD) is characterized by proteinaceous aggregates named Lewy Bodies and Lewy Neurites, and the small protein α-synuclein (α-syn) is the main component of these aggregates. Amyloid protein co-aggregation with lipid membranes is an unexplored aspect, potentially associated with toxicity and propagation of PD.
Our goal is to understand the mechanism and specificity of co-aggregation using model membranes with different lipid components and compositions. Explore the influence of lipid membranes on the aggregation of α-synuclein (α-syn), structure and lipid composition of the co-aggregates.
Our results reveal a selective co-aggregation, as well as, a dependence of the aggregation rate on lipid composition, pH and charge. These studies take advantage of well characterized and reproducible aggregation kinetics. Cryo-TEM images uncovered different fibrillar structures. Identification and a qualitative approximation to lipid composition on the co-aggregates were revealed by NMR and phosphorus assays.

L-934

Current induced potential oscillation in planar lipid bilayers

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The lipid-protein-matrix of biological membranes can be understood as excitable media that react sensitive to alteration of physico-chemical parameters. It was shown that phenomenons like oscillation and pattern formation can occur spontaneously. Here we show that voltage-oscillation-frequencys in different lipid-protein-setups with a small pore forming peptide alamethicin depend on the physical state of the membrane.

L-935

Lipids and LHCII - the tale of two entities

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The photosythetic membrane of chloroplast consists of multiple entities: lipids, polar and non-polar ones, photosynthetic reaction centres, electron transporters and many others and it must conduct many biochemical reactions that have to be regulated. It is vital for propper membrane functioning that all membranes' elements to interoperate.
We tried to focused only on two entities: the light harvesting pigment-protein antenna complex of photosystem II (LHCII) and plant galactolipids such as MGDG, DGDG and PG. The aim of this work is to determine mechanisms and types of interactions between LHCII and its lipid surrounding and compare it with native thylakoids membranes with different lipid compositions. To achieve this goal we used several spectroscopic methods like infrared spectroscopy, low-temperature fluorescence and fluorescence lifetime measurements.
Spectroscopic data showed the type of protein-protein and lipid-protein interactions during the membrane stacking. Examination of the type of interactions observed in an artificial, less complicated system makes mechanisms of specific thylakoid membrane *in vivo* organization foreseeable.
Acknowledgements KG acknowledges the National Science Centre, Poland for financial support - FUGA2 grant no 2013/08/S/NZ1/00823.

Abstracts
– 22. Protein-Lipid Interactions –

L-936

Detection of conformational change in the autoinhibitory segments of ESCRT-III proteins

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ESCRT-III (endosomal sorting complex required for transport) proteins mediate membrane invagination and constriction in many bilayer remodelling processes including sorting of ubiquitinylated membrane proteins, cytokinesis, plasma membrane repair, nuclear envelope reformation, and viral budding. ESCRT-III proteins cycle between soluble monomeric and membrane-associated multimeric states, with the transition being regulated by membrane-bound proteins and cytosolic factors including Vps4 ATPases. The carboxy terminus in ESCRT-III encompasses two helices which harbour recognition sites for binding factors and also act as autoinhibitory segments. They fold back onto the protein core and prevent protein-lipid interactions but are thought to be released in the ESCRT-III multimer.
We aim at detecting rearrangements of the two C-terminal alpha-helices in ESCRT-III. For this purpose we fluorescently double-labelled human ESCRT-III member CHMP3 to observe displacement of C-terminal segments *in vitro* via a change in FRET efficiency. In ensemble fluorescence experiments we address conformational changes in response to ESCRT-III multimer formation and membrane association, using human CHMP2A protein and lipid vesicles. Further steps will see the addition of protein binding partners.

L-937

Membrane interaction of the glycosyltransferase WaaG

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The glycosyltransferase WaaG is involved in the synthesis of lipopolysaccharides in Gram-negative bacteria. It was identified as a potential antibiotic target and inhibitor scaffolds were studied. It is located at the cytosolic side of the inner membrane.
In the crystal structure an exposed and largely α-helical 30 residue peptide containing a net positive charge was identified as a putative membrane interacting region (MIR-WaaG) of WaaG. We studied its membrane interaction by fluorescence quenching, circular dichroism, and solution state NMR.
Upon interaction with anionic vesicles MIR-WaaG adopts a largely α-helical structure. From quenching studies with hydrophilic and lipophilic quenchers we find that MIR-WaaG binds to anionic vesicles. We solved the NMR-structure of MIR-WaaG in micelles and found that it agrees well with the crystal structure. Using paramagnetic agents we find that the N- and C-terminal residues of MIR-WaaG are surface exposed while a central part of the peptide is immersed in the micelles. All tyrosines are located at the interface between hydrophobic and hydrophilic environments.
We conclude that the membrane interaction of WaaG with *E. coli* inner membranes is at least in parts conferred by MIR-WaaG and electrostatic interactions play a key role in binding.

L-938

Biologic activity and interactions of cationic peptides derived from cecropin D with membrane models

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Antimicrobial peptides (AMPs) are essential components of the innate immunity and are considered promising alternatives to conventional antibiotics. In this study, the neutral peptide Cecropin D-like *G. mellonella* (WT) was used as framework to study the effect of increasing charge on antimicrobial and cytotoxic activity of two modified peptides, M1 and M2, with charges of +5 and +9, respectively. The results exhibit that increases of the charge enhances the activity against *Gram*-negative bacteria in contrast to WT peptide, which does not have antimicrobial activity. Cytotoxic experiments in erythrocytes showed that the charge is correlated with hemolytic activity. It was also measured the release of Calcein from Small Unilamellar Vesicles (SUVs) composed of mixtures of POPG, POPC and POPE phospholipids. The results show that all the peptides permeabilize POPG membranes, whereas in others SUVs, only M1 and M2 induced permeabilization. The interaction of peptides with phospholipids at the level of the glycerol backbone and hydrophobic domain was studied using Laurdan and DPH probes, respectively. The results suggest that peptides induce an ordering effect in DMPG and DMPC:DMPG SUVs.

L-939

Oxidized phospholipids modify pore forming activity of Bax protein in mitochondrial membranes

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Key regulator of apoptotic process is the Bcl-2 protein family whose members meet at the mitochondrial outer membrane (MOM) and arbitrate a life or death (membrane permeabilization) decision there. The main protein causing permeabilization is the apoptotic Bax protein which upon stress-induced activation translocate to the MOM, inhibits there the pro-survival Bcl-2 protein, and induces pore formation (1). Recently it was found, that the mitochondrial membrane system seems to play an active role with its lipids directly involved. It was namely found that under oxidative stress conditions oxidized lipids can be generated that are directly involved in mitochondrial apoptosis (2). Addition of oxidized phospholipids into MOM mimicking model lipid bilayer enhanced membrane affinity and partial penetration of full length Bax (3). Using fluorescence leakage method we found that Bax induced leakage which was sensitive to the type and concentration of OxPL in the membrane.
(1) B. Leber et al, *Apoptosis* 12 (2007), 897.
(2) G.O.Fruehwirth et al, *BBA* 1772 (2007), 718.
(3) M. Wallgren et al, *BBA Biomembranes* 1818 (2012), 2718.

Abstracts

– 23. Molecular Recognition –

L-940

Binding of glycosaminoglycans to interleukin-10 studied by NMR spectroscopy

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The protein interleukin (IL)-10 is a key regulator of the cellular immune system, which prevents an overwhelming immune reaction and tissue damage. IL-10 is bound by glycosaminoglycans (GAGs) – a class of highly sulfated polysaccharides in the extracellular matrix that play a decisive role in the biology of many cytokines e.g. for receptor binding or protection from proteolytic degradation. Knowledge of the structural principles guiding the interaction between GAGs and IL-10 is very limited but is mandatory in order to understand its biological function. However, structure determination of protein-carbohydrate complexes is often impeded by a weak ligand binding affinity and the paucity of non-exchangeable protons at close distances to the protein as requirement for a NOE-based NMR structure determination. Here, we used a combination of ligand-detected NMR methods (STD, transferred NOESY) along with experiments based on protein- and ligand-immobilized paramagnetic spin labels to determine the structure of the GAG-IL-10-complex. Our results suggest binding of GAGs to a cluster of basic amino acid residues at the central crevice of the IL-10 dimer and provide a hypothesis for how GAGs can regulate IL-10's biological activity.

L-941

The antigenicity and dynamics of foot-and-mouth disease virus capsids from southern Africa

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Foot-and-mouth disease (FMD) is a disease that affects even-toed hoofed mammals. The FMD virus (FMDV) is the causative agent of FMD, of which the South African Territories (SAT1, 2 and 3) serotypes show a high degree of antigenic diversity. Vaccination with one virus may not provide immune protection from the other SAT strains. The identification of B-cell epitopes is therefore key to the design of high-crossover vaccines. The first aim was to employ *in silico* epitope prediction programmes to predict B-cell epitopes on SAT capsids. Secondly, the dynamics of the immunodominant GH loop among the SAT serotypes were to be investigated to further aid in understanding its antigenic structure. Homology models of 18 immunologically distinct SAT protomers were built and input to two epitope prediction servers, *Discotope1.0* and *Ellipro*. Residues predicted by both programmes were defined as epitopes. Also, three models representative of the SAT serotypes were subjected to molecular dynamics. Both experimentally characterised and novel epitopes were predicted. A putative novel antigenic site was also identified. The molecular dynamics revealed a novel conformation of the GH loop in one SAT3 strain, which may be attributed to a unique disulphide bond anchoring it.

L-942

Explaining TIR1 molecular recognition via tomographic docking

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Molecular docking is a widely applied method for the initial screening stages in drug discovery. It allows to make predictions about the binding affinity and to compare the interaction strength of different ligands and receptors. We develop a new, fully general, docking approach, which we call tomographic docking, that uses autodock-vina as its core engine, and allows high-resolution scans of binding pockets and channels. We apply our method to the TIR1 receptor-ligand system, which is characterized by a high selectivity of the binding site for both natural and synthetics ligands. Our results show that geometrical and physical-chemical constraints within the binding pocket are responsible for the selectivity of the receptor and the interaction strength of ligands. Moreover, the detected constraints help in identifying which structural features in both receptor and ligands are responsible for non-covalent association, thus providing key insight in the mechanism of auxin perception.

Abstracts
– 24. Neurosciences –

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Solitary electromechanical pulses in axons

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Investigations of nerve activity have focused predominantly on electrical phenomena. It is to be expected that the state of the nerve cell depend not only on electrochemical potentials and the conjugated flux of ions but also on all other thermodynamic forces including variations in lateral pressure and temperature. In the past, experiments by Iwasa & Tasaki demonstrated small changes in nerve thickness and length during the action potential (1). While both mechanical and thermal signals are very small, they are found to be in phase with voltage changes (2). Such findings have led to the suggestion that the action potential may be related to electromechanical solitons traveling without dissipation (3). A condition for the existence of such a soliton is the existence of an order transition in the membrane from solid to liquid lightly below physiological temperature. Here, we present ultrasensitive AFM recordings of mechanical changes on the order of 0.2 – 1.2 nm in the giant axons of the lobster. In a recent publication we showed that action potentials traveling in opposite direction in some nerves pass through each other upon collision (4). Also, when stimulated at opposite ends of the same axon, colliding action potentials pass through one another and do not annihilate. These observations are consistent with a mechanical interpretation of the nervous impulse.

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The molecular anatomy of dendritic spines

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How memory is stored within the brain is still enigmatic. One hypothesis is that synaptic strength is modulated by changing the protein composition in the postsynaptic dendritic spines. Although the functions of many of these proteins have been described in detail in the synapse, an overall understanding on how information is stored is still missing. This is largely due to the fact that the molecular anatomy of the dendritic spine, meaning the nanoscale localization and the quantity of the different proteins, is unknown. Here, we use a combination of super-resolution imaging, mass spectrometry and electron microscopy to describe the nanoscale localization of postsynaptic proteins and their abundance. We investigate 150 proteins in this way, ranging from neurotransmitter receptor organization, scaffolding proteins, ion channels and kinases. We found that proteins involved in the same physiological processes are correlating very closely, for example CaMKII and calmodulin. In total the combined techniques will enable us to create a realistic 3D model of the dendritic spine, which we can use to simulate physiological processes like ion fluxes in this complex compartment.

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Effects of varenicline and nicotine on native α3β4 nicotinic ACh receptors in human chromaffin cells

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We assessed electrophysiologically the activities of varenicline on alpha3beta4 nAChRs expressed in human adrenal chromaffin cells from organ donors and compared its effects with those of nicotine. Under voltage-clamp conditions, both varenicline and nicotine evoked whole-cell currents in these cells but varenicline was more potent and efficacious than nicotine. In current-clamp mode, stimulation of the cells with 10 ms puffs of acetylcholine (ACh) (300 μM) evoked action potentials (APs) that were sensitive to inhibition by the sodium channel antagonist tetrodotoxin. Perfusion of 50, 100, 250, and 500 nM varenicline in a step-wise manner over a 5 min period robustly increased the number of APs fired whereas nicotine did not. However, perfusion of nicotine (50 nM) tripled the number of ACh-evoked APs when perfused together with varenicline (100 nM). Our results demonstrate that varenicline and nicotine increase the excitability of adrenal chromaffin cells which may alter the release of catecholamines from these cells.

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The influence of the microenvironment's stiffness on the physiology of Schwann cells and neurons

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Schwann cells are of particular physiological importance for the peripheral nervous system function. They form the myelin around the axons of peripheral nerves that enables the physiologically necessary high conduction velocity of nerves. In addition, Schwann cells are involved in the secretion of the extracellular matrix which provides essential mechanical support to peripheral nerves. Schwann cells are exposed to a mechanical environment whose stiffness significantly changes along the morphogenesis of peripheral nerves. There is increasing evidence that diverse cell types are able to feel and respond to the stiffness of their microenvironment, and that this ability is of particular physiological importance. We hypothesized that Schwann cells are able to feel and respond to the stiffness of their microenvironment and that this ability is essential for their diverse physiological functions. To test this hypothesis we established a primary Schwann cells-neurons co-culture and designed an extracellular matrix with tunable stiffness values. Our preliminary data indicate that Schwann cells feel the stiffness of the microenvironment and that the stiffness determines their morphology. In the next step we will study their physiological response comprehensively.

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The molecular organisation of the synaptic bouton

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We present here the 3D model of a synaptic bouton. It is composed of more than 300,000 individual proteins as absolute copy numbers of 60 different proteins that are essential to synaptic vesicle recycling. We used quantitative immunoblotting to determine protein copy numbers, electron microscopy to measure organelle numbers, morphology and distribution, and super-resolution fluorescence microscopy (STED) to localise the proteins within the synaptic bouton. Using quantitative mass spectrometry, we estimated the copy numbers of >1100 additional proteins. The copy numbers of proteins involved in the same step of synaptic vesicle recycling correlate closely, while copy numbers vary over more than three orders of magnitude between steps. Synaptic vesicle exocytosis is amply provided for, with more than 20,000 copies of each exocytotic SNARE per synaptic bouton. Proteins involved in synaptic vesicle endocytosis, on the other hand, are far less abundant (∼1000-4000 copies each) and sufficient for the simultaneous recycling of only ∼7-11% of all vesicles in the average synaptic bouton.

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