

Collection of Poster Abstracts

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Protein Structure, Dynamics, and Function

P1. Actin induced activation of the T3SS effector YopO studied by PELDOR spectroscopy

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The bacterium *Yersinia pestis* is the causative agent of plague. *Yersinia* have evolved an impressive set of tools to defend themselves against attacks by the human immune system. For example, *Yersinia* are able to avoid phagocytosis by injecting a set of six Yop proteins (Yersinia outer proteins) into attacking macrophages by utilizing their syringe-like type-three-secretion-system (T3SS). Inside the macrophage, the Yop proteins interfere with several important cellular processes. As a result, the macrophage is no longer able to engulf and destroy the bacterium and instead even protects it from further attack (1). One of the Yop proteins, YopO (also known as YpkA), is subject of this presentation. When injected into the host cell, YopO specifically interferes with the regulation of the actin cytoskeleton in at least three different ways: 1) The C-terminus of YopO binds to Rac1, an actin cytoskeleton regulating GTPase and acts as a guanidine nucleotide dissociation inhibitor. 2) YopO binds to monomeric actin, forming a stable 1:1 complex. This interaction with actin leads to autophosphorylation and activation of the N-terminal kinase domain of YopO. 3) The bound actin molecule is then used as a bait to recruit and phosphorylate various cellular targets that are involved in cytoskeletal dynamics.(2, 3)

To investigate the actin induced activation of YopO, we have conducted detailed PELDOR distance measurements and cw-EPR experiments on the spin labelled YopO/Actin complex. We use trilateration to follow the domain movements and found that the activation of YopO by actin is a complicated process that involves major structural rearrangements.

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P2. Fast Folding Dynamics of an Intermediate State in RNase H Measured by Single-Molecule FRET

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We have studied the folding kinetics of the core intermediate (I) state of RNase H by using a combination of single-molecule FRET (smFRET) and hidden Markov model analysis. To measure fast dynamics in thermal equilibrium as a function of the concentration of the denaturant GdmCl, a special FRET labeled variant, RNase H 60-113, which is sensitive to folding of the protein core, was immobilized on PEGylated surfaces. Conformational transitions between the unfolded (U) state and the I state could be described by a two-state model within our experimental time resolution, with millisecond mean residence times. The I state population was always a minority species in the entire accessible range of denaturant concentrations. By introducing the measured free energy differences between the U and I states as constraints in global fits of the GdmCl dependence of FRET histograms of a differently labeled RNase H variant (RNase H 3-135), we were able to reveal the free energy differences and, thus, population ratios of all three macroscopic state ensembles, U, I and F (folded state) as a function of denaturant concentration.

P3. Dynamic cholesterol-conditioned dimerization of CXCR4

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G protein coupled receptors (GPCRs) allow for the transmission of signals across biological membranes. For a number of GPCRs, this signaling was shown to be coupled to prior dimerization of

the receptor. Consequently, modulation of signaling may be achieved either by chemical modification of the dimer interface or by changes in the lipid-protein interaction. The functionality of the chemokine receptor type 4 (CXCR4) was reported before to depend on membrane cholesterol and receptor association.

Here, we address the dimerization pattern of CXCR4 both in pure phospholipid bilayers and in cholesterol-rich membranes. Using ensembles of molecular dynamics simulations, we show that CXCR4 dimerizes promiscuously in phospholipid membranes. Addition of cholesterol dramatically affects the dimerization pattern: cholesterol binding largely abolishes the preferred dimer motif observed for pure phospholipid bilayers with transmembrane helices 1 and 7 (TM1/TM5-7) at the dimer interface. In turn, the symmetrical TM3,4/TM3,4 interface is enabled first by intercalating cholesterol molecules. These data provide a molecular basis for the modulation of GPCR activity by its lipid environment.

P4. Modeling the voltage-gated proton channel NpHv1 in both open and closed states

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The Hv1 voltage-gated proton channel plays an important role as part of the cellular proton extrusion machinery. Hv1 has been identified in a wide range of eukaryotes throughout the animal kingdom, with the exception of insects. Very recently, we reported the existence of an HV1-type proton channel in *Nicotia glauca* (NpHv1), an insect of the Zygentoma order [1]. NpHv1 comprises 239 amino acids and is 33 % identical to the human proton channel hHv1.

In this work, we report homology modeling of the structurally unknown NpHv1 using the crystal structures of the voltage-sensing domains (VSD) of *Ciona intestinalis* (Ci-VSD) in putatively open and closed conformations [2] as templates. Next to a careful alignment procedure, we generated multiple models of NpHv1 in both open and closed states, selected one final model of NpHv1 in each state, and subsequently submitted the final NpHv1 models to all-atom molecular-dynamics (MD) simulations. We analyzed the interaction network between the voltage-sensing residues Arg157, Arg160 and Arg163 in S4 and the proton selectivity filter Asp66 in S1. The solvent accessibility of selected residues of our models was compared with experimental data and found to be in good agreement. Moreover, comparative analysis of both open and closed state models suggests a possible structural explanation for the experimental observation that Trp207 in hHv1 (Trp159 in NpHv1) adopts a key role in gating kinetics [3].

Our MD-refined models represent our first approach to create a structural framework. These models may be used to better understand the biophysical properties of NpHv1 and voltage-gated proton channels in general.

P5. Advancing Fluorescent Proteins for Super-resolution Microscopy

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Super-resolution fluorescence microscopy is the method of choice to monitor cellular and subcellular biological processes in live cells. Among the different fluorescent labels presently available, fluorescent proteins (FPs) of the GFP family have the key advantage of being genetically encodable. Localization-based super-resolution microscopy approaches require photoactivatable FPs (PA-FPs) that will change their spectral properties upon irradiation with light of a particular wavelength. To be able to distinguish individual, activated fluorophores from the background and to localize them with high precision, a high photon yield in the activated state and a high dynamic range, i.e., the contrast ratio between the fluorescence of the activated (on) and deactivated (off) states, are essential. In stimulated emission depletion (STED) super-resolution microscopy, the sample is raster-scanned by a tightly focused excitation beam followed by a red-shifted, donut-shaped depletion beam. Any FP used for STED must be exquisitely photostable because it has to go through multiple excitation-depletion cycles while the sample is scanned near its location. Moreover, it must be excitable and de-excitable by the lasers that are typically installed in commercial STED microscopes. Therefore, far-red emitting

FPs are preferred.

We have selected the green-to-red photoconvertible mEosFPthermo and the far-red emitting mGarnet as templates for targeted protein engineering. Considering that FPs are all very similar and share the same scaffold, an obvious strategy was to identify specific amino acid residues that elicit certain properties to one FP and introduce the corresponding amino acid in the other FP variant by using site-directed mutagenesis. As we will show, such simple rational engineering approaches often do not meet with success, which clearly shows that our current understanding of the physics of proteins is far from being complete.

P6. Global Substrate Dynamics Provides a Rationale for Perturbed γ -Secretase Cleavage Patterns

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A key step in the development of Alzheimer's disease (AD) is the enzymatic cleavage of the Alzheimer Precursor Protein (APP) transmembrane domain (TMD) by γ -secretase (GSEC). GSEC is located in the cell membrane and cleavage of the TMD leads to the formation of A β -peptides (1). A subset of these A β -peptides with lengths of 42 or 43 amino acids forms A β -plaques in the brain. Their ratio to the non-toxic A β 40 peptide is of relevance for occurrence of AD. Single point mutations in the TMD sequence, the familial AD (FAD) mutations, shift the ratio between A β 42/A β 43 and A β 40 towards higher A β 42/A β 43 levels, leading to early onset AD. We study the dynamics of the TMD of wildtype and FAD mutants by molecular dynamics simulations. Our results revealed several dynamic motifs along the TMD: A dynamical N-terminal domain is connected by a hinge to a rigid C-terminal domain harbouring the cleavage sites. We showed the impact of synthetic mutations on the global dynamics of the APP TMD (2). Recently, we investigated seven FAD mutants. Surprisingly, the C-terminal cleavage site was not affected. However, the collective dynamics revealed significant differences in the location and extent of the previously described hinge region. Furthermore, higher order modes revealed a second hinge region close to the initial cleavage sites, which is also shifted by FAD mutations. The collective dynamic is highly correlated with the backbone hydrogen bond network. FAD mutants perturb this network by breaking interconnects between residues. This interaction network might be the key for substrate positioning in the active site of GSEC and therefore provides a rationale for the observed shifts in the A β ratios through differences in substrate positioning. This is in good agreement with experimental investigations targeting GSEC dynamics (3).

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P7. The role of glycans in viral infections: binding studies followed by native mass spectrometry

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Glycans are highly discussed as attachment factors in viral infection pathways nowadays. The surface of target cells is decorated with many glycans[2]. Currently, the mechanism of host protein glycan interaction in viral attachment and cell entry is less understood. Specific glycans on human cells seem to be necessary as viral attachment factors which increase the susceptibility to infections. Noroviruses (NoV) were used as model system to study glycan binding with native mass spectrometry (MS)[3]. This powerful technique allows the investigation of proteins in their native state. Non-covalent interactions are still intact[4].

The main structure of interest for glycan interaction is the protruding (P) domain of NoV which forms dimers and is a part of the major capsid protein VP1[3]. In order to study the attachment mechanism between viruses and host cells, we analyzed the binding of various glycans, especially human histo blood group antigens (HBGA). We compared the glycan binding pattern from two different GII.4 human NoV strains. A GII.4 crystal structure with HBGA B saccharides shows that two equivalent glycan binding sites per dimer exist[2]. To get more detailed information about this interaction we

varied the glycan concentration and type. STD-NMR and co-crystallization experiments of P dimers bound to glycans were carried out for comparison[1].

Recorded data strongly suggest that cooperative multi step binding plays a major role in viral cell attachment and entry. MS data clearly reveal four instead of two glycan binding sites on the P dimer[1]. Furthermore, received MS data show a glycan mediated structural change in the whole P domain. Moreover, distinct NoV strains indicate preference for different glycan ligands.

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P8. Combining biochemical and biophysical approaches to study non-covalent PAR-protein interactions

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The post-translational modification poly(ADP-ribosylation) (PARylation), which is mainly catalyzed by the enzyme poly(ADP-ribose) polymerase 1 (PARP1), is one of the first signaling events occurring upon DNA damage. It regulates specific DNA repair pathways, chromatin remodeling and gene transcription and therefore fulfils important functions during the maintenance of genomic integrity. Besides covalent modification of proteins, proteins can also interact in a non-covalent manner with PAR. Previous studies revealed that PAR binding to proteins regulates macromolecular complex formation in a PAR-chain length dependent manner with strong consequences on protein functionality (1-4). However, little is known about the mechanism of this interaction on the molecular level. We investigate the consequences of non-covalent PAR-protein interactions on the three-dimensional structure and dynamics of proteins as well as of PAR. This includes the examination of the effect of chain length and branching frequency of the polymer. Besides several biochemical methods like size exclusion chromatography and electrophoretic mobility shift assays, especially, Fourier transformed infrared spectroscopy (FTIR spectroscopy) is employed to gain insights into the binding mechanism. The tumor suppressor protein p53 presents the focus of investigations as it exhibits high affinity to PAR and forms distinct complexes dependent on chain length (4).

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P9. Structural Characterization of a T1SS in Salmonella using Electron Microscopy

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A type 1 secretion system (T1SS) was identified recently in *Salmonella enterica* that is necessary for invasion of polarized epithelial cells. This T1SS is encoded by the *Salmonella* pathogenic Island 4 (SPI4) and consists of the SiiAB-complex, an outer membrane pore (SiiC), a periplasmic adaptor protein (SiiD), a giant non-fimbrial adhesion protein which consist of 53 bacterial immunoglobulin domains (SiiE) and the inner membrane ATP-binding cassette (SiiF) (1, 2).

Little is known of the SiiAB complex. It displays a weak homology to MotAB, which forms a proton channel through the inner cell membrane and functions as a stator molecule in the flagellum (3). However, since the complete structure of both complexes remain unknown it is our goal to get

structural insights into the SiiAB and MotAB complexes to gain further knowledge of its function. Here we show preliminary results of purified SiiAB and MotAB using negative stain electron microscopy.

We also present preliminary results of a Cryo-Transmission Electron Tomography (Cryo-TEM)-study of *Salmonella enterica*. We produced *Salmonella* minicells and have attached a gold conjugated antibody to the non-fimbrial adhesin to locate the T1SS in the membrane. We show first results of subtomogram averaging of the T1SS in the Membrane.

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P10. Links between structure, dynamics and function for proteins functionalized at nano-carbon platforms

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Reox-active proteins can be readily functionalized at diversely modified conducting platforms (electrodes) by using alkanethiol-based self-assembled monolayer films (SAMs) or other carbon-based nanostructures. Application of the modern, temperature- and high-pressure-assisted voltammetry techniques furnished by variation of the SAM thickness, SAM terminal group composition and solution composition, allows not only for the identification of electron transfer (exchange) mechanism (e.g., tunneling vs. dynamically controlled regimes), but also for drawing conclusions about the degree of protein's interfacial confinement and its further stabilization/destabilization by the solution additives. Two cases of the environs' remarkable impact will be discussed: (A) Combined effect of the surface confinement and medium's vitrification on azurin, latter caused by the high concentration of organic salt, choline dihydrogen phosphate. We disclosed essential structural stabilization of azurin, along with the dynamically controlled ET mechanism combined with a remarkable nonergodic aspect [1,2]. (B) Essential impact of a strength of the protein-SAM interaction (free diffusion vs. nonflexible docking) on the structural stability and intrinsic ET mechanism for the Fe(III/II)H₂O form of myoglobin. In this case, change of protein's stability and conformational flexibility was found to be correlated with a degree of the inner-sphere reorganization of a Fe-coordinated water, hence with the rate of electron transfer [3,4]. (C) Quasi-simultaneous proton-coupled two-electron transfer and direct wiring for glucose-oxidase, pierced by the carbon nanotube-polymer hybrids [5] will also be discussed.

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P11. Data-driven Langevin Modeling of Protein Dynamics

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The in silico prediction of rare biomolecular events represents a long-standing problem, because slow (say, mil- lisecond timescale) processes are typically out of reach of current all-atom molecular dynamics (MD) simulations. To circumvent this time scale problem, one often invokes massive parallel computing strategies or various enhanced sampling techniques such as replica exchange, metadynamics etc. While these approaches may provide an efficient sampling of the system's free energy landscape, a dynamical description requires some coarse-grained "post-simulation" model that

is capable of rebuilding the kinetics from the sampled data. To this end, we have recently proposed a data-driven Langevin equation (dLE) approach (1) that constructs a low-dimensional dynamical model from a given MD trajectory. Here we present a methodology to combine the dLE with metadynamics simulations. Employing the metadynamics trajectory for initial sampling of the free energy landscape a number of starting configurations for subsequent short MD trajectories are chosen, which generate input data for the dLE model. Adopting simple but nontrivial example of hierarchical structural dynamics of a peptide helix we compare the conformational transition rates between standard MD simulations and dLE. We show that this approach reproduces the peptide dynamics in standard MD simulations albeit with a significant reduction in computational effort. Finally, we report on the convergence measures and virtues of this approach.

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P12. The thermodynamics and molecular interactions of PcTx1 with the acid sensing ion channel 1a

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The acid sensing ion channel 1a (ASIC1a) is a proton gated channel involved in many physiological processes and drug target for treating neurological disorder as well as inflammatory and neuropathic pain. PcTx1, a 40-residue peptide isolated from the venom of the tarantula *Psalmacabras cabridgei*, is the most potent selective inhibitor of ASIC1a. Recent crystal structures of the ASIC1a-PcTx1 complex have revealed an extensive network of peptide-channel contacts. Our subsequent study combining simulations and mutagenesis of the peptide and the channel revealed that only a subset of the many contacts in the ASIC1a-PcTx1 crystal structure are critical for PcTx1 activity, thus defining the main pharmacophore of PcTx1. Despite the large amount of structural and functional information on the binding of PcTx1 to ASIC1a, little is known about the thermodynamics and pathway of binding.

This presentation will describe the results from the first extensive simulation study of a peptide-ASIC1a complex. The potential of mean force (PMF) of the binding of PcTx1 from/to the ASIC1a-PcTx1 complex was reconstructed from a series of umbrella simulations using both umbrella integration (UI) and the Weighted Histogram Analysis Methods (WHAM) method. The predicted binding affinities (ΔG) from the two methods are in good agreement with data from experimental binding studies. Furthermore, results indicate that electrostatic steering dominates the pathway of binding and suggest that certain peptide-interactions are functionally important but make less contribution to the overall binding affinity than other contacts. This has implications for the design of peptide mimetics to inhibit ASIC1a.

As part of this study we have also developed a set of freely available python scripts for the analysis of umbrella simulations that include error analysis and convergence checks.

P13. Investigating the Ezrin-mediated attachment of F-actin networks

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The shape, mechanical stability and driving forces of cells are highly dependent on their cytoskeleton. Actin is one of the main proteins which contributes to this biological network. The mechanical movements of the cell rely on the interaction of filamentous actin (F actin) with several other proteins (1). One of these proteins is myosin II which belongs to the superfamily of myosin motor proteins. By hydrolysing ATP myosin is able to walk along F-actin or to induce tension on these filaments (2).

In our model system F actin networks are attached to pore spanning lipid bilayers (PSLBs) via electrostatic interactions or the linker protein ezrin which mimics biological situation. Ezrin has a Phosphatidylinositol-4,5-bisphosphate (PIP2) binding site located at the N-terminus and a F-actin binding site at the C-terminus and is therefore responsible for the linkage of F-actin to PIP2 present in the PSLB. Several actin binding proteins and cross-linkers are introduced during the polymerization of actin filaments. The variations of the self-organization on lipid bilayers are observed via confocal laser

scanning microscopy.

Besides the recorded self-organization, the mechanical properties of different F-actin networks are examined. Atomic force microscopy is used to determine the lateral membrane tension of the PSLB dependent on different F-actin and actin myosin, so called actomyosin, networks. The viscoelastic properties of the network will be recorded by passive microrheology using the mean square displacement of a polymer beads Brownian movement. The polymer bead is locked up inside the different networks during the polymerization.

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P14. What vibrations tell us about GTPases

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We performed intensive studies on small (1) and heterotrimeric (2) GTPases using experimental and theoretical infrared spectroscopy. From the time-resolved FTIR spectra in combination with site-directed mutagenesis and isotopic labelling we obtained detailed insights into the reactions and conformational changes of the proteins. We monitored the order of events during the GTPase reactions and found new reaction intermediates (1).

The vibrational spectrum is very sensitive with respect to changes in geometry and charge shifts. For example, a change in bond length of 0.001 Å can be resolved by a resolution of 4 cm⁻¹. Changes on this scale are already important for catalysis. By means of biomolecular simulations this information can be decoded from the experimental spectrum. We found e.g. that binding of GTP to Ras and GAP induces an eclipsed conformation for the GTP that plays an important role in catalysis (3).

Using the ATR (attenuated total reflection) technique, membrane tethered GTPases can be investigated in a flow through system and protein-ligand, protein-protein and protein-drug interactions can be studied marker-free. We found that Ras, which plays a major role in cancer, can dimerize at the membrane and we suggest a dimerization interface. This is a new target for anti-cancer drugs (4).

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P15. Comparative Study of the Nuclear Egress Complexes of Several Herpes Viruses Using MD Simulations

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Herpes is a viral disease that can be fatal in immunocompromised individuals. A promising target for the treatment of herpes is the nuclear egress complex (NEC), which is essential for virus replication. The recently published crystal structures of the heterodimeric NECs of three different herpesviruses display a high structural similarity even for distantly related viruses (1,2). In order to assess the role of individual amino acids and of a zinc ion for complex formation, molecular dynamics (MD) simulations were performed. The initial results indicate that the complexes exhibit a similar overall dynamics and display a similar energy profile of the interacting residues. In addition, the unliganded NEC proteins were studied in the presence and absence of zinc to assess the role of ion binding on conformational sampling prior to complex formation. Ultimately, we hope to facilitate the design of ligands that interfere with NEC formation of a broad spectrum of herpesviruses.

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P16. The cysteine SH-stretching mode as a site-specific IR probe for conformational change in proteins

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The cysteine SH-stretching mode causes an IR absorption band with useful features: it lies in a “clean” range of the spectrum near 2550 1/cm, where no other protein components absorb, and its precise location and shape depend on the protein environment. We employ these features to monitor the redox-induced exposure of the C-terminal membrane anchor in the flavoprotein pyruvate oxidase from *E. coli* (EcPOX).(1) We studied the thiol band of EcPOXwt, cysteine knockout mutants, and EcPOXd23, where the membrane anchor (23 residues) had been removed. Redox-triggered FT-IR difference spectra were recorded with a thin-layer spectroelectrochemical reflection cell.

The absolute IR absorption spectra of EcPOXwt and -d23 show a similar, distinct and composite thiol band. While typically weak and broad due to heterogeneous solvent interactions, these strong thiol bands indicate rigid H-bond environments.(2) The oxidized-minus-reduced IR difference spectra of both proteins, however, are dissimilar. In EcPOXwt, the difference spectrum is dominated by contributions from the protein backbone, reflecting major conformational changes. Moreover, a rarely seen S-shaped thiol difference band indicates changing thiol environments. In EcPOXd23, the protein backbone signals are weak, and the thiol difference band completely disappears, even though all ten cysteines are still in place. We could assign the thiol difference band to C88 and C494, both of which are remote from the moving C-terminus. These cysteines sense a propagating conformational change triggered by the C-terminal exposure.

Strategically placed cysteines could help to pinpoint structural rearrangements and to trace signal transduction pathways in proteins.

(1) Russell P, Hager LP, Gennis RB. (1977) JBC. 252:7877–82.

(2) Bare GH, Alben JO, Bromberg PA. (1975) Biochem. 14:1578–83.

P17. Conformational changes in the substrate binding protein of a TRAP transporter from *V. cholerae*

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Tripartite ATP-independent periplasmic (TRAP) transporters are an important group of membrane transporters in bacteria and archaea. They are composed of two transmembrane domains and a periplasmic substrate binding protein (SBP). The SBP scavenges the substrate (e.g. sialic acids), undergoes conformational change and delivers it to the transporter. Release of the substrate reverses the SBP into its unbound, open conformation. The molecular details of the interaction between the SBP and the domains are still unknown and the exact mechanism of the transporting process is still part of current studies.

However, for an efficient transport process, only the substrate loaded (closed) SBP should bind to the transporter. It is thus important to investigate whether the conformational changes of the SBP are strictly substrate induced or if the molecule constantly samples the open-, closed- or possibly intermediate conformations.

We aim to answer this question using EPR distance measurements on the SBP SiaP from *V. cholerae*. We used the “difference distance matrix” feature of mtsslWizard to find optimal spin labeling positions on the molecular surface of SiaP. The amino acid residues of SiaP were then mutated in pairs to cysteines at the optimal positions and then labeled with MTSSL. High quality PELDOR data of the spin-labeled SBP were recorded in the presence and absence of its substrate (Neu5Ac).

Furthermore, X-ray structures of SBP homologues propose that the substrate binding is due to a salt bridge between the residues Arg127, Glu184 and His207. This assumed salt bridge was disturbed by the introduction of further mutations and PELDOR data was recorded for the new SiaP variants. Our results reveal new insights into the conformational changes of the SBP in frozen solution and the interactions which are critical for substrate binding.

P18. DNA Deformation of Wildtype and Mutated Binding Sequences of CcpA and AmtR

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Selective binding of transcription factors (TFs) to DNA is an essential part of cellular life. In order to fulfil their diverse functions, TFs display a specificity that ranges from one discrete binding sequence to a number of similar sequences which share common features. To which degree, and in which way, this specificity is conferred by different shapes and different deformability of the different DNA sequences is the focus of on-going research. We used molecular dynamics simulations to investigate the differences between wildtype binding-sequences and designed mutant-sequences of the TFs CcpA and AmtR.

AmtR binds the DNA as a homodimer with each head contacting four bases leaving a gap of six non-contacted bases in between. In order to assess the role of shape readout in the AmtR system, the central six bases of the wildtype sequence have been replaced either by more or by less kinkable base steps. Both mutant sequences exhibit a higher internal energy than the wild type indicating a strained conformation. The easily kinkable mutant sequence can form all base contacts seen in the wild type and has a comparable KD, while the less kinkable mutant sequence is missing some contacts and has a higher KD.

CcpA binds as homodimer and kinks the DNA at the central base pair step of the sequence. We compared the dynamics and energetics of three binding sequences and assessed the effect of three different mutants of the central base step. The simulations show a comparable fluctuation of the DNA kink in all wild type simulations. The mutant sequences are kinked in the same range as the wild type sequences, but the internal energy difference between free and bound form indicates a higher cost for mutant DNA deformation compared to wild type DNA deformation.

P19. Binding of antagonist caffeine to human adenosine receptor hA2AR in nearly physiological conditions

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Lipid composition may significantly affect membrane proteins function, yet its impact on the protein structural determinants is not well understood. Here we present a comparative molecular dynamics (MD) study of the human adenosine receptor type 2A (hA2AR) in complex with caffeine—a system of high neuro-pharmacological relevance—within different membrane types. These are POPC, mixed POPC/POPE and cholesterol-rich membranes. 0.8- μ s MD simulations unambiguously show that the helical folding of the amphipathic helix 8 depends on membrane contents. Most importantly, the distinct cholesterol binding into the cleft between helix 1 and 2 stabilizes a specific caffeine-binding pose against others visited during the simulation. Hence, cholesterol presence (~33%-50% in synaptic membrane in central nervous system), often neglected in X-ray determination of membrane proteins, affects the population of the ligand binding poses. We conclude that including a correct description of neuronal membranes may be very important for computer-aided design of ligands targeting hA2AR and possibly other GPCRs.

P20. Proteolytic activation of a latent recombinant polyphenol oxidase from wine leaves

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Polyphenol oxidases (PPOs) are type-3 copper proteins and catalyze the hydroxylation and oxidation of mono- and diphenols to o-quinons. These o-quinons then react autocatalytically to melanin. Latent plant polyphenol oxidases consist of a N-terminal domain carrying the catalytic site and a C-terminal domain, which are connected by a short linker unit. In the latent state, the C-terminal domain is discussed to shield the access to the active site for phenolic substrates. Latent PPOs can be activated by conformational changes of the linker and the C-terminal domain, induced by amphiphilic reagents such as fatty acids or sodium dodecylsulphate (SDS). Less is known about activation by proteolytic

cleavage of the C-terminal domain as it may happen in vivo. A prominent characteristic of plant PPOs is the presence of a highly conserved phenylalanine (F259), which is localized in immediate vicinity to the entrance of the catalytic pocket. This bulky gate residue is discussed to regulate the access to the active site for phenolic substrates.

We expressed recombinant L-VvPPOcs-2wt (the latent PPO isoform 2 from *Vitis vinifera* Cabernet Sauvignon) and a mutein (L-VvPPOcs-2-F259L) in *E. coli*. Both convert mono- and diphenols after activation with SDS. The mutein L-VvPPOcs-2-F259L shows a significant lower activity than the wildtype. We investigate different proteases for proteolytic activation. Only trypsin leads to an efficient activation. As revealed by mass spectrometry the cleavage site of trypsin is located in the linker region. Proteolytic activated L-VvPPOcs-2wt and L-VvPPOcs-2-F259L show a higher and especially similar activity. Our experiments may give new insights into the role of the gate residue in latent and activated plant PPO.

P21. Modeling of the Transmembrane Domain of the IgM-BCR Complex

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The functionality and therefore correct assembly of B cell receptors (BCR) is crucial for signal transduction and triggering of a proper immune response. The BCRs of all major immunoglobulin subclasses are composed of a transmembrane-bound immunoglobulin of a certain isotype (mIgA, mIgD, mIgE, mIgG or mIgM) and the Ig- α /Ig- β heterodimer. Moreover, the transmembrane domain (TMD) of each immunoglobulin consists of two specific, identical chains (e.g. two μ heavy chains in the case of mIgM). The TMD of the BCR was reported to play a key role in activation (1). Despite considerable interest in the mechanisms of BCR activation, little is known about the structure of the TMD.

Here, we explore the assembly and the structure of the full IgM-BCR-TMD, which is the result of a tripartite dimerization process. The different dimerization configurations of two components of the IgM-BCR-TMD were analyzed inside phospholipid bilayers using coarse-grained simulations.

(1) J. Yang, M. Reth. (2010) *Nature*. Vol: 465–469

P22. Navigating Protein Conformation Spaces by Kino-Geometric Sampling and Modulating Frustrated Motions

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Proteins exchange between different states to perform their biological function (1). Despite experimental, structural snapshots of conformational states, understanding how proteins use collective motions to transition between them remains a formidable challenge. We present our all-atom Kino-Geometric Sampling (KGS) framework for multi-level analysis of protein conformational landscapes. KGS represents proteins as kinematic linkages, with dihedral angles as degrees of freedom and non-covalent interactions and non-native contacts as holonomic constraints. Motions on the constraint manifold naturally encode collective motions of degrees of freedom. KGS efficiently performs instantaneous rigidity and mobility analysis (2), motion-planning based exploration of conformation space, and has characterized excited conformational substates from sparse data. We recently introduced dynamic Clash-avoiding Constraints (dCC) that instantaneously redirect collective motions towards conformations with favorable energies. dCC modulate frustrated motions, trading internal energy (enthalpy) for protein flexibility (entropy), which enable KGS to navigate rugged conformational landscapes between substates. We present examples of large and small-scale conformational transitions, and evaluate our method against peer methods. By analyzing propagated motions, we found a previously uncharacterized allosteric network of residues in cyclophilin A, connecting the active site to a recently proposed, non-canonical capsid binding site 25Å away. This network is validated by Carr-Purcell-Meiboom-Gill (CPMG) experiments and multi-temperature crystallography, rationalizing experimental data from various resources in one structural basis. In all, KGS is an

efficient all-atom method suitable to reveal detailed, physiological molecular mechanisms for small and large amplitude motions on multiple levels.

(1) van den Bedem, Fraser. (2015) Nat. Meth. 12(4):307–318.

(2) Budday et al. (2015) JMPS. 83:36–47.

P23. Study The Substrate binding to the OEC and mechanism of water oxidation at RT by fs-XFEL

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Photosynthetic water oxidation in green plants, algae and cyanobacteria is catalyzed by the Mn₄CaO₅ cluster associated with the Photosystem II core complex (PSII_{cc}), a multi-subunit membrane protein complex. In the last decade, the electronic and geometric structures of this light-driven water splitting catalyst have been extensively investigated. Until now, the dynamic mechanism of the water oxidation reaction at the Mn₄CaO₅ cluster is still elusive. Conventional synchrotron X-ray radiation crystallography was used over years to determine the structure of PSII_{cc} from cyanobacteria, which always implies radiation damage of the Mn₄CaO₅ cluster to a certain extent. In contrast, employing femtosecond X-ray diffraction on PSII_{cc} microcrystals by using a free electron laser provides the possibility for recording the diffraction data at room temperature (RT) before the onset of radiation damage. The crystal structures of Photosystem II at ambient temperature were obtained for the illuminated S₃-state at 2.8 Å and the dark-adapted S₁-state at 3.0 Å resolution. Distinct differences in the overall structure compared to the reported cryogenic temperature structures are observed; higher side-chain mobility with multiple conformers, expansion of the dimer in the membrane plane with changes in the helix orientations, and longer cofactor-cofactor distances. There were no major structural changes observed between the dark and illuminated state, which precludes the mechanisms that require large changes in the S₃ state. To determine the O-O bond formation mechanism in the OEC, the binding site of the substrate water on the Mn₄CaO₅ cluster must be determined. NH₃, a water analog, known to bind to the Mn₄CaO₅ cluster in the S₂ and S₃ states can be used to distinguish substrate from non-substrate waters. Here we are discussing the different classes of the suggested water oxidation mechanisms depending on our RT crystallographic data in combine with EPR and EXAFS data.

P24. Pharmacology of Binary Toxins: Blockage of Protective Antigen

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Background: Several intracellular acting bacterial protein toxins of the AB-type, which are known to enter cells by endocytosis, are shown to produce channels. This holds true for protective antigen (PA), the binding component of the tripartite anthrax-toxin of *Bacillus anthracis*. Evidence has been presented that translocation of the enzymatic components of anthrax-toxin across the endosomal membrane of target cells and channel formation by the heptameric PA₆₃ binding/translocation component are related phenomena. Chloroquine and some 4-aminoquinolones, known as potent

drugs against *Plasmodium falciparum* infection of humans, block efficiently the PA63-channel in a dose dependent way.

Methodology/Principal Findings: Here we demonstrate that related positively charged heterocyclic azolopyridinium salts block the PA63-channel in the μM range, when both, inhibitor and PA63 are added to the same side of the membrane, the cis-side, which corresponds to the lumen of acidified endosomal vesicles of target cells. Noise-analysis allowed the study of the kinetics of the plug formation by the heterocycles.

Conclusions/Significance: These results strongly argue in favor of a transport of lethal factor through the PA63-channel and suggest that the heterocycles used in this study could represent attractive candidates for development of novel therapeutic strategies against anthrax.

Beitzinger, C., et al. 2013. Designed azolopyridinium salts block protective antigen pores in vitro and protect cells from anthrax toxin. *PLoS One*. :e66099.

Bronnhuber, A., et al. 2014. Inhibitions of the translocation pore of *Clostridium botulinum* C2 toxin by tailored azolopyridinium salts protects human cells from intoxication. *Toxicology*. 316:25-33.

P25. EHD2 restrains dynamics of caveolae by an ATP-dependent, membrane-bound, open conformation

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The EH-domain containing protein 2 (EHD2) is a dynamin-related ATPase that confines caveolae to the cell surface by restricting the scission and subsequent endocytosis of these membrane pits. For this, EHD2 is thought to first bind to the membrane, then to oligomerize, and finally to detach, in a stringently regulated mechanistic cycle. It is still unclear how ATP is used in this process and if membrane binding is coupled to conformational changes in the protein. Here, we show that the regulatory N-terminal residues and the EH-domain keep the EHD2 dimer in an autoinhibited conformation in solution. By significantly advancing the use of infrared reflection-absorption spectroscopy, we demonstrate that EHD2 adopts an open conformation by tilting the helical domains upon membrane binding. We show that ATP-binding enables partial insertion of EHD2 into the membrane, where G-domain-mediated oligomerization occurs. ATP hydrolysis is related to detachment of EHD2 from the membrane. Finally, we demonstrate that the regulation of EHD2-oligomerization in a membrane-bound state is crucial to restrict caveolae dynamics in cells.

New Techniques and Challenges in Biophysics

P26. New Ultrarapid-Scanning Interferometer for FT-IR spectroscopy with Microsecond Time-resolution

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A novel Fourier-transform infrared (FT-IR) rapid-scan spectrometer has been developed which yields 1000 times higher time resolution as compared to conventional rapid-scanning spectrometers. The central element to achieve faster scanning rates is based on a sonotrode whose front face represents the movable mirror of the interferometer. A prototype spectrometer with a time resolution of 13 μ s was realized, capable of fully automated long-term measurements with a flow cell for liquid samples. The performance of this novel spectrometer is demonstrated by recording the photoreaction of bacteriorhodopsin initiated by a short

laser pulse that is synchronized to the data recording. The spectrometer allows for future investigations of fast, non-repetitive processes, whose investigation is challenging to step-scan FT-IR spectroscopy.

P27. Traction force microscopy for tumor spheroids in 3D collagen gels

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Cell movements and associated cell-generated forces are of central importance during embryogenesis, wound healing, or cancer metastasis. In the latter case, individual tumor cells leave a primary tumor and invade interstitial tissue to metastasize. To enable this invasion process, cells exert physical forces on the extra-cellular matrix. Recent advances have made it possible to quantify the contractile forces of single cells within highly non-linear materials like collagen gels (1). However, many tumor cell lines are known to show collective invasion dynamics. Furthermore, the structure of the tissue may be remodeled by contractile forces of the tumor. To study the physical forces arising from these collective effects, we embed MDA-MB-231 breast tumor and HT-1080 fibrosarcoma spheroids containing several thousand cells in reconstituted collagen. As the tumor grows and cells spread into the surrounding tissue, the collagen matrix is deformed. We measure the collagen deformation by tracking embedded silica beads in the equatorial plane of the spheroids. The displacements fields are fitted to a non-linear material model to infer the contractile forces. During the early phases of tumor growth (<6h), the collagen displacements are highly asymmetric but with time become radially symmetric. In addition, contractile forces increase linearly with time while cells at the border of the spheroid move outwards. For comparison, we measure a non-invasive cell line (MCF-7) and also find significant contractile forces but no cells leave the spheroid. Instead, the initially irregular-shaped MCF-7 spheroids attain a round shape over time. This finding at the population-level is in line with a recent report (2) that contractile forces of individual cells are not sufficient to account for differences in cell invasiveness between metastasizing and non-metastasizing tumor cells.

(1) Steinwachs J, et al. (2015) Nature methods. 13:171-176

(2) Koch TM, et al. (2012) PLoS ONE. 7(3): e33476

P28. Towards unidirectional reconstitution of membrane proteins into liposomes.

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Functional studies of membrane proteins (MP) in their native environment (the cellular membrane) is challenging due to the complexity of the native membranes and measurement interferences from other membrane constituents or reactions. Therefore, MPs are often purified and integrated into systems which mimic their natural environment in a membrane as e.g. liposomes or nanodiscs, a process called reconstitution, allowing to investigate its function and structural aspects without any disturbing

background.

The most important problem during MP-reconstitution is the often random orientation of the MP in the liposomal membrane after reconstitution. For functional studies of the MP of interest and quantitative analysis of its properties, unidirectional orientation in the liposomal membrane is required. Previous work of other groups, where MPs have been reconstituted and studied, did not include a final and universal approach (Rigaud JL, Levy D. (2003) *Methods Enzymol.* 372:65-86.; Tunuguntla R et al. (2013) *Biophys. J.* 105(6):1388-96.) and procedures have to be individually optimized for an enrichment of enzyme orientation. In most cases, however, orientation cannot be influenced and is thought to depend on the three-dimensional structure of the protein (Tunuguntla R et al. (2013) *Biophys. J.* 105(6):1388-96; Nordlund G et al. (2014) *Nat. Commun.* 5:4303.).

We are currently developing and establishing a universal method to force unidirectional reconstitution of MPs by the aid of a molecular unit that can be attached to every protein. Our method is based on the observation that the F1F0 ATP synthase with its large hydrophilic head piece (F1 part) orients highly uniformly when embedded in preformed vesicles. We try to mimic this situation with a bioengineering approach that should be applicable to a wide variety of proteins. Once such a method is fully established, the interplay of two or more membrane proteins can be investigated more quantitatively.

P29. Variability and reproducibility of cell mechanical measurements with microfluidic systems

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We describe a quantitative high-precision, high-throughput method for measuring the mechanical properties of cells in suspension with a microfluidic device consisting of a parallel array of micron-sized constrictions. Using a high-speed (750 frames/s) CCD camera, we measure the speed, deformation and entry time into microconstrictions of several hundred cells per minute. Cell entry time into microconstrictions decreases with increasing driving pressure and decreasing strain (maximum cell compression inside constriction) according to a power-law. From this power-law relationship, the cell elasticity and fluidity can be estimated. Here, we systematically analyze the origin of variability between cell mechanical measurements with this microconstriction setup. We find that stress and strain stiffening largely determine the variability between measurements in which cell stress (driving pressure) and cell strain (max. deformation) are not identical. We compensate for these systematic influences of pressure and strain using a histogram matching method. With this method, we investigate the influence of cell culture conditions and measurement details. We find e.g. a significant stiffening of cells with increasing time after cell harvesting. Lastly, we measure cells that transiently overexpress the GFP-tagged nuclear envelope protein lamin A. We can reliably quantify the influence of lamin A expression levels on cell mechanics by extending our histogram matching method. We take the fluorescent intensity of each cell as an additional sorting parameter and can therefore correlate the expression level of lamin A with cell mechanical properties. Together, our findings demonstrate that histogram matching of pressure, strain and protein expression levels greatly reduces the variability between measurements and enables us to reproducibly measure small differences in cell mechanical properties between different groups of cells with unprecedented high precision.

P30. Exploring molecular landscapes inside cells with in situ cryo-electron tomography

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In situ cryo-electron tomography was used to investigate the native molecular landscape of *Chlamydomonas* cells. Vitreous cells were thinned by focused ion beam milling and subsequently imaged by cryo-electron tomography, allowing the reconstruction of 3D views of the cell in its native

state. The molecular resolution of these tomograms enabled detailed structural and functional analysis of the molecular environment in various organelles.

Within the nucleus, we found most of the proteasomes accumulating in clusters at the inner membrane of the nuclear envelope, especially around nuclear pores. Subtomogram classification was able to distinguish assembly and functional states on a single-protein level, and also revealed a population of proteasomes bound to the inner membrane by a linker protein. Combining the precise positions of the protein complexes within the cell with their functional states, the different subpopulations could be analyzed statistically.

Besides structural and statistical analysis, we were also able to detect novel structures. We discovered protein arrays within the narrow center regions of the trans-Golgi cisternae. Due to their linking function, they aid in maintaining the narrow luminal space, therefore likely directing cargo protein traffic to the trans-Golgi periphery.

Lastly, we are also able to directly visualize protein organization within the chloroplast. Using 2D projections along the normal of the thylakoid membrane, we were able to visualize the photosynthetic proteins along the varying architecture of stacked and unstacked thylakoid membranes. Mapping them back into the thylakoids, we gained insights into how thylakoid structure directs light harvesting.

Here, we present the very first steps towards Visual Proteomics, with the goal of mapping individual molecular complexes back into the cellular volume, allowing the investigation of each macromolecule's structure, function, and interaction with its native environment.

P31. Supramolecular Tandem Membrane Assays - A Facile Way to Monitor Membrane Transport

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Supramolecular tandem assays utilize reporter pairs composed of macrocyclic, supramolecular receptors and fluorescent dyes to monitor dynamic concentration changes. We have previously shown that this affords a versatile method to monitor enzyme activity, which is suitable to be scaled up to high-throughput screening (1). A new application of the supramolecular tandem assay principle emerges from encapsulation of the reporter pairs inside liposomes (2).

Herein, we will show that the resulting supramolecular tandem membrane assays allow unambiguous detection of membrane transport of organic molecules across the lipid bilayer membrane by various transport mechanisms including passive diffusion (permeation), which is otherwise challenging to detect. This affords a simple method to determine membrane permeability coefficients and activation energies as reference data for optimization and computational studies.

(1) M. Schnurr, J. Sloniec-Myszk, J. Döpfert, L. Schröder, A. Hennig. (2015) *Angew. Chem. Int. Ed.* 54:13444-13447; R. N. Dsouza, A. Hennig, W. M. Nau. (2012) *Chem. Eur. J.* 18:3444-3459; A. Hennig, H. Bakirci, W. M. Nau. (2007) *Nat. Methods* 4:629-632.

(2) G. Ghale et al. (2014) *Angew. Chem. Int. Ed.* 53:2762-2765.

P32. Redox Microscopy: A sensitive method to quantify production and degradation of H₂O₂ from single cells

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Hydrogen peroxide (H₂O₂) oxidizes intracellular target molecules, thereby controlling cellular signaling. However, quantification and sensitivity to determine production and degradation of H₂O₂ from single cells are limited. Using an electrochemical setup (redox microscope) and applying different voltammetric techniques, with a bare disk platinum ultramicroelectrode (10 μm; vs Ag/AgCl), very low [H₂O₂] could be resolved at plasma membrane production sites of single cells: 2 nmol/l (square wave voltammetry), 50 nmol/l (cyclic and linear scan voltammetry) and 500 nmol/l (chronoamperometry, CA). Although offering the lowest sensitivity for H₂O₂ CA measurements are unbeatable for long-term determinations with high temporal resolution (≥ 1 Hz). From single human monocytes, average H₂O₂

production was 1.5 nmol//s over 60 minutes following stimulation with the phorbol ester TPA. During the initial phase (25 min) rate was 3.4 nmol//s (n=23). Considering quantitatively the concomitant H₂O₂ degradation by the same cell, net production rates reached 9.0 nmol//s. Single cell measurements were validated in human monocyte populations by electron spin resonance spectroscopy and an adapted fluorescence-based Amplex® UltraRed assay. In summary, physiologically relevant low nanomolar [H₂O₂] can be spatially and temporally resolved direct at the H₂O₂ production sites of single cells.

P33. The Effects of Preparation and Location on mid-infrared Spectra of Skin recorded in vivo

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Diabetes Mellitus, the inability to properly regulate one's blood glucose levels, requires the regular measurement of glucose from an invasively acquired drop of blood. The current uncomfortable measurement procedure leads diabetics to refrain from measuring as often as necessary to keep blood glucose levels around normal values. In order to alleviate the shortcomings of the current method photoacoustic and photothermal detection methods of mid-infrared spectra of skin in-vivo have been proposed (1,2). Calibrating such spectra with skin requires knowledge of the components and behavior of skin at different locations in-vivo. With photoacoustic and photothermal detection the suitability of different locations on the human hand for the correlation of IR spectra with blood glucose levels are investigated. Moreover one can follow the changes of skin in-vivo after removing skin lipids with soap. These results show that the choice of measurement location and pre-measurement treatment have a major influence on the suitability of IR-spectra for correlation with blood glucose for a continuous non-invasive measurement system.

Protein Folding, Assembly, and Stability

P34. Site-specific dynamics of β -sheet peptides probed by laser-excited temperature-jump IR spectroscopy

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We investigated the influence of turn residues and side-chain interactions of model β -sheet peptides by time-resolved temperature-jump infrared spectroscopy. The conformational dynamics of two-stranded β -hairpins and three-stranded β -sheets were analyzed using a quantum cascade IR laser setup in combination with a laser excited T-jump (1). D-Pro-Gly turn sequences which favor a tight β -turn (2) are used to guarantee the stability of the model peptides in aqueous solutions. Furthermore the Xxx-D-Pro tertiary amide provides a resolved IR band (3), allowing us to probe the turn dynamics site-specifically. In addition, folding can be assisted by aromatic cross-strand interactions. The contribution of different forces to the nucleation of a hairpin in the β -sheet folding process was reflected by differences in the T-jump relaxation kinetics. Relaxation times after the T-jump occur on a sub-microsecond timescale and were monitored as a function of temperature to obtain activation energies. It was found that aromatic interactions between the strands slow down the folding process as a hydrophobic collapse is involved. Solvent molecules have to be displaced, structural rearrangements occur, and interactions of the hydrophobic residues must be formed.

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(2) L. Wu, D. McElheny, V. Setnicka, J. Hilario, and T.A. Keiderling. (2012) Proteins: Structure, Function, and Bioinformatics. 80: 44-60

(3) S.V. Kuznetsov, J. Hilario, T.A. Keiderling, and A. Ansari. (2003) Biochemistry. 42: 4321-4332

P35. Filament formation of actin via coarse grained MD simulations.

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Actin is one of the most abundant proteins in eukaryotic cells. Its importance lies in the ability to form polymer strands, the actin filaments, which are one of the main component of the cytoskeleton network. This network is of major importance for several biological functions like the mechanical stability of the cell, its organization and cell motility. The latter process is thought to be originated in the "treadmilling" reaction, in which a single actin strand is growing along one end while dissembling on the other. This process involves the consumption of ATP and is related to a conformational change of the actin units.

Monomer units of actin already consists of a sequence 375 aminoacids which makes atomistic computer simulations of an actin filaments very expensive. Here we present our recently developed coarse grained model of actin based on the MARTINI forcefield (1). The model obtained is found to be in good agreement with the results of atomistic simulations. We further show some preliminary results of a screening of the interaction of two actin monomers via the DAFT method (2). This procedure offers first microscopic insights in the formation of filaments and their preferred binding geometries. In summary, this results extends MARTINI simulations to the realm of the cytoskeleton and enables future studies of the interplay between this network and other cellular components.

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(2) T. A. Wassenaar et al.. (2015) J. Chem. Theory Comput. 11:2278-2291.

P36. In-situ folding observation by SEIRAS of cell-free expressed bacteriorhodopsin into nanodiscs.

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The functional correct insertion, folding and assembly of functional proteins in biological membranes are essential for processes and activity of a cell. We present Surface-enhanced absorption spectroscopy (SEIRAS) as a novel method for monitoring membrane protein folding into artificial lipid bilayers during cell-free protein expression. A tethered bilayer, supported by a SEIRAS active gold surface, serves as a lipidic folding milieu for newly synthesized membrane proteins. Cell-free expression of the model retinal protein bacteriorhodopsin (bR) as well as rhomboid protease GlpG and disulfide bond formation protein B (DsbB) takes place in the bulk solution. The time resolved SEIRAS exclusively observe phenomena on the gold surface, and hence provide structural information of the folding dynamics at atomic/molecular level

occurring in the artificial membrane. For bR, analysis of the obtained spectra reveals three phases at the lipid surface after gene transcription and translation have been initiated: pre-conditioning period, a lag phase to cleanse preoccupied contaminant on the lipid surface; phase I, first folding stage, in which newly synthesized protein is inserted into the tethered lipid bilayer and gradually forms secondary structures; phase II, second folding stage in which the secondary structure containing protein gains tertiary structure.

Single Molecule Biophysics

P37. 2D kinetics of single domain antibodies binding tumor markers

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Therapeutic antibodies are now a common treatment of major diseases, especially in cancer. While capable to bind soluble antigens, antibodies do often bind their target at the interface between an immune cell and a target cell or a pathogen surface. Affinity, describing the «strength» of binding, is the ratio of association and dissociation rates which characterizes bond formation and breaking. While affinity between these molecules is usually measured with one reactant in solution (i.e., three dimensions environment or 3D), these conditions do not take into account the physical aspects at cell-cell interface (i.e., 2D) that include force and relative motion of molecules constrained at surfaces. Our purpose is to correlate 2D affinity and kinetics measurements with cellular response. To quantify 2D binding properties, we perform kinetic measurements of five single domain antibodies (sdAbs) against the tumor marker HER-2 using a Laminar Flow Chamber (LFC). In the LFC, HER-2 is coated to a microbead surface and interacts with sdAbs bound to the chamber bottom surface in the presence of flow. This allows us to measure at single molecular level the association kinetics (as function of number of bond formed and of total interaction time between molecules) and the dissociation kinetics (as bonds lifetimes) under forces in piconewton range. Results show changes in 2D kinetic parameters depending on applied force and molecular contact time, and strong differences of affinity comparing with 3D measurements. Our sdAbs may be used to form bi-specific antibodies for immune cell recruitment. We will perform tumor cell killing experiments with bi-specific antibodies recruiting NK cells through an anti-CD16 sdAb and recruiting target tumor cells through anti-HER-2 sdAb, and measure target killing as a function of 2D binding properties. This study provide a physical characterization of antigen-antibody interactions and can be useful for the selection of antibodies in therapeutics.

P38. The nature of a hard protein corona forming on quantum dots exposed to human blood serum

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Biological responses of cells and organisms to nanoparticle exposure crucially depend on the properties of the protein adsorption layer (“protein corona”) forming on nanoparticle surfaces. Their characterization is a crucial step toward a deep, mechanistic understanding of their formation. Previously, we have systematically studied adsorption of one type of model protein on nanoparticles in situ by using fluorescence correlation spectroscopy. Here, we present the first such study of interactions between water-solubilized CdSe/ZnS quantum dots (QDs) and a complex biofluid, human blood serum. Despite the large number of proteins in serum, we observed a protein layer of well-defined (average) thickness forming on QD surfaces. Both the thickness and the apparent binding affinity depended on the type of QD surface ligand. Kinetic experiments revealed that the protein corona formed from serum is irreversibly bound, whereas the one formed from human serum albumin was earlier observed to be reversible. By using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectrometry, we identified the most abundant serum proteins contributing to the formation of a hard corona on our QDs.

P39. Coiled Coils as Mechanical Building Blocks

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Coiled coils (CCs) are important structural motifs found in many different proteins. They are made of α -helices that self-assemble into helical superstructures such as dimers, trimers and tetramers. In Nature CCs are important components of cytoskeletal and extracellular matrix proteins, indicating their importance as critical mechanical building blocks. Despite their widespread appearance in these structural proteins, little is known about their mechanical properties. This knowledge is crucial for controlling the folding and stability of individual CCs, and more importantly for understanding the mechanical properties of biological tissues.

With the goal of shedding light on the structure-to-mechanics relationship of CCs, we are applying AFM-based single molecule force spectroscopy to obtain the force threshold for CC dissociation. We are currently investigating the effects of sequence, length and pulling geometry on CC stability. We show that a 4-heptad CC ruptures at a most probable force of >40 pN when loaded in 'shear' geometry, while the rupture force for the 'unzip' geometry is below the detection limit of AFM. In the 'shear' geometry we further observe a clear dependence on CC length. For example, a 3-heptad CC ruptures at significantly lower forces of ~ 30 pN. Our final goal is to develop a library of CCs for the synthesis of CC-based materials with tunable mechanical properties for applications in tissue engineering.

P40. Interplay between a fluctuating membrane and the stochastic protein binding

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Biological membranes connect to adjacent cells or the extra-cellular matrix with the help of adhesion proteins forming bonds. These bonds transmit stresses across cell boundaries and pin the membrane locally. This in turn affects the mechanics of the fluctuating membrane. Here, we investigate the static and dynamic mechanical properties of a membrane upon stochastic formation of a single bond in a minimal model system by theoretical means and compare our results to detailed simulations. We calculate the eigenmodes of the pinned membrane to determine its static properties and show that the pinning can be treated by an effective potential. For the membrane dynamics of a pinned membrane, we introduce a first order correction to the damping coefficients of the unbound membrane to recover the power spectral density obtained in simulations.

In a next step, we account for stochastic association and dissociation of the bond and determine effective reaction dynamics. The latter integrates the thermally excited membrane and protein fluctuations, and calculate the effects of opening and closing of a bond on the fluctuation spectrum of the membrane.

In summary, we provide a full picture for the mechanical effects of a single bond binding to a flexible membrane. Our results provide a tool-box for studying non-thermal effects in membranes allowing for the identification of parameters affected by the "active" part of the system.

P41. Origins and consequences of pausing during bacterial transcription initiation

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Gene transcription is the first and most important step in gene expression and regulation. Transcription is performed by RNA polymerases, a multi-subunit enzyme that synthesizes full length RNA from a

DNA template. In bacteria, the core RNA polymerase assembles with one of the σ initiation factors to form the RNA polymerase holoenzyme (RNAP) that recognizes specifically gene promoters, which are DNA sequences close to transcription start sites. Once the RNAP-promoter complex is formed, RNAP opens the promoter, synthesizes the nascent RNA by scrunching downstream DNA into itself and, eventually, releases the interactions with the promoter to escape into the elongation phase of transcription. The protein escape transition is highly regulated by promoter sequence which, this way, controls the expression levels of a given gene.

Using single-molecule FRET and gel assays, we show here that initial transcription is halted for ~ 10 s by a DNA-sequence-dependent pause at the +6 position (i.e., 6 nucleotides downstream from the starting point of transcription). We also show that the lifetime of the pause at +6 is modulated by the presence of a triphosphate at the 5'-end of the RNA, suggesting that direct interactions between the 5'-RNA end and the σ factor 3.2 subdomain assist the nascent RNA synthesis. Furthermore, we observe that the initial pause at +6 is able to isomerize into a long-lived pause state (~ 50 s), with a probability of isomerization depending on the concentration of NTPs, the promoter DNA sequence at +6 and the 5'-RNA end nature. Our work shows that a complex network of interactions between RNAP, the 5'-end of the RNA and the downstream DNA sequence at the +6 position control the yield of successful promoter escape as a function of NTP concentration, and, therefore, regulate gene expression levels.

P42. Adhesion behavior of malaria infected red blood cells studied by AFM force spectroscopy

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Tropical malaria caused by the parasitic protozoan *Plasmodium falciparum* is the most severe form of all malaria infections. During maturation in infected red blood cells (iRBCs), *P. falciparum* builds up a structure on the iRBC-surface composed of adhesion receptors localized in so-called knobs, which allow the iRBCs to adhere to the endothelium. Through this strategy the parasite avoids clearance by the spleen, and thus increases its chance to survive in the host. However the enhanced adhesion leads to inflammation of the endothelium and several complications like pregnancy-associated malaria (PAM). In PAM the infected red blood cells bind via the membrane protein VAR2CSA to the receptor chondroitin sulfate A (CSA) in the intervillous space of the placenta. This leads to an aggregation of the iRBCs preventing the exchange of nutrients between mother and child. Recently our group has shown that the bond between these two receptors might be explained by a catch bond behavior (1). In our current project we aim to characterize the bond between VAR2CSA and CSA in more detail to gain a better understanding of VAR2CSA-CSA binding mechanism. Therefore atomic force microscopy (AFM) force spectroscopy is used to measure the binding force of isolated VAR2CSA and CSA depending on temperature and pulling speed. Furthermore we will characterize the adhesion of whole iRBCs to CSA functionalized surfaces and cantilevers.

(1) Rieger, H., Yoshikawa, H. Y., Quadt, K., Nielsen, M. A., Sanchez, C. P., Salanti, A., Tanaka, M. & Lanzer, M. (2015) Cytoadhesion of *Plasmodium falciparum*-infected erythrocytes to chondroitin-4-sulfate is cooperative and shear enhanced, *Blood*. 125, 383-391.

P43. Investigations into the protonation dynamics of cytochrome c oxidase

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Fluorescence correlation spectroscopy (FCS) is a single molecule based technique to temporally resolve rate-dependent processes by correlating the fluorescence fluctuations of individual molecules traversing through a confocal volume. In addition, chemical processes like protonation or intersystem crossing can be monitored in the sub-microsecond range. FCS thereby provides an excellent tool for investigations of protonation dynamics in proton pumps like cytochrome c oxidase (CcO). To achieve this, the pH-dependent fluorescent dye fluorescein was attached as a protonation probe to the CcO surface via site-specific labeling of single reactive cysteines that are located close to the entry point of a proton input channel (K-pathway). The analysis of protonation dynamics is complicated by

overlapping triplet and protonation rates of the fluorophore. A Monte Carlo simulation based algorithm was developed to facilitate discrimination of these temporally overlapping processes thus allowing for improved protonation reaction rate determination. Using this simulation guided approach we determined precise local proton association and dissociation rates and provide information about protein surface effects, such as proton collecting antennae, on the transport properties of proton transfer channels.

P44. Translocation versus binding in the recognition of short oligonucleotides by a biological nanopore

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Polymer interactions with pore-forming membrane proteins, as evidenced by the resulting block of ionic current, are of two types: (1) the threading of an extended polymer chain of which only a small length (2) a binding reaction, where the polymer enters the pore entirely and interacts as a particle. The latter phenomenon typically occurs in dilute aqueous electrolyte solutions (e.g. KCl < 1 M) and requires long and charged molecules and can allow sequencing (e.g. of DNA). In contrast, the binding reaction typically occurs with short, neutral polymer molecules, requires high salinity (e.g. KCl 3-4 M) and enables the high-resolution discrimination of polymer masses¹.

The aerolysin nanopore has recently been shown to strongly interact with short adenine oligonucleotides (A3-A10) and this interaction allows mass discrimination on the basis of the depth of block of ionic current induced by the binding of the analyte², suggesting a binding-type interaction. Using low-noise current recording, we have identified strong dynamics of this interaction between DC and 50 kHz and identified short visits to deeper blocked states preceding and following the principal, mass-dependent state (pre- and post-blocks). Statistical analysis indicates that the probability of post- but not pre-blocks decreases with (1) oligomer length and (2) transmembrane voltage. We interpret this finding in terms of a combined translocation and binding interaction, probably involving several binding sites for DNA in the pore.

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(2) Cao et al. Nature Nanotechnology 2016, 1–7.

P45. Functional reconstitution of integral membrane proteins in polymer supported membranes.

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Functional reconstitution of integral membrane proteins (IMPs) in lipid model systems which ensure two-dimensional mobility and biological activity for advanced fluorescence microscopy techniques would be highly beneficial. Towards this end we have developed polymer supported membranes (PSM) based on glass substrates which are modified with a poly (ethylene glycol) brush carrying hydrophobic palmitic acid headgroups for the capture and subsequent fusion of proteoliposomes into contiguous and defect free membranes. Mobility of reconstituted lipids and IMPs was confirmed by FRAP and single molecule localization and tracking. We successfully employed PSMs for the quantification of ligand-induced dimerization of reconstituted IMPs and systematically studied homo- and hetero-oligomerization of E.coli β -barrel outer membrane proteins and archaeal α -helical IMPs, which are in perfect agreement with in vivo and in silico data (1).

(1) Rassam P., Copeland NA., Birkholz O., Toth C., Chavent M., Duncan AL., Cross SJ., Housden NG., Kaminska R., Seger U., Quinn DM., Garrod TJ., Sansom MSP., Piehler J., Baumann CG., Kleanthous C. (2015) Nature. 523:333-336

P46. High-speed single particle tracking on model lipid membranes

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Interferometric scattering detection microscopy (iSCAT) is a powerful method for single particle tracking (SPT) experiments. Recently, we reported on the use of iSCAT for visualizing the diffusion of gold nanoparticles (GNPs) as small as 5nm attached to lipids in model membranes with nanometer lateral precision and at up to 1 MHz frame rate (1). Exploiting this high spatial and temporal resolution, we revealed transient nanoscopic confinements of nanometer size on millisecond time scales on supported lipid bilayers (SLBs) (2). To avoid artifacts caused by the glass-lipid interface, we have extended our studies to pore-spanning membranes (PSMs) and to giant unilamellar vesicles (GUVs). We prepare PSMs over 5µm sized holes by GUV spreading and directly compare the diffusion on the free-standing membrane with the diffusion on the supported parts between the pores. GUVs as three-dimensional model membrane system offer an entirely substrate-free platform. Here, we demonstrate one of the unique capabilities of iSCAT, namely high axial resolution in tracking the displacement of a nanoparticle. We present three-dimensional trajectories of GNP-labeled lipids and unlabeled virus-like particles diffusing on a GUV membrane.

(1) S. Spindler, J. Ehrig, K. König, T. Nowak, M. Piliarik, H. E. Stein, R. W. Taylor, E. Garanger, S. Lecommandoux, I. D. Alves, V. Sandoghdar, J. Phys. D: Appl. Phys. 49 (2016)

(2) C.-L. Hsieh, S. Spindler, J. Ehrig, V. Sandoghdar, J. Phys. Chem. B 118 (2014).

P47. Conservation and divergence in nucleotide excision repair lesion recognition

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Nucleotide excision repair (NER) is an important, highly conserved DNA repair mechanism with an exceptionally large range of chemically and structurally unrelated targets. Lesion verification is believed to be achieved by the helicases UvrB and XPD in the prokaryotic and eukaryotic processes, respectively. Using single molecule atomic force microscopy (AFM) analyses, we demonstrate that UvrB and XPD are able to load onto DNA and pursue lesion verification in the absence of the initial lesion detection proteins. Interestingly, our studies show different lesion recognition strategies for the two functionally homologous helicases, as apparent from their distinct DNA strand preferences, which can be rationalized from the different structural features and interactions with other NER protein factors of the two enzymes.

P48. Probing mechanical properties of nucleic acids with magnetic tweezers: the torsional stiffness of DNA

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DNA is the carrier of the genetic code and central to all cellular life. The read-out and processing of the (sometimes) highly-packed DNA is regulated by its mechanical properties and interactions with proteins. Small deformations from the nucleic's equilibrium, can be described by the isotropic elastic rod model, including bending, stretching and twisting.

Magnetic tweezers (MT) can probe the response of single molecules to external forces and torques. In MT, the molecule of interest, here DNA, is tethered between a glass cover slip and a magnetic bead. By controlling the position and rotation of permanent magnets, above the flow cell, forces and torques can be applied to stretch and twist the DNA tether. Forces in a range of 0.1 pN up to 100 pN can be reached. In particular also small forces (< 1 pN), characteristic of non-covalent macromolecular interactions, can readily be applied and measured. Furthermore, our MT setup is able to track multiple beads (currently up to ~60) at the same time, enabling the collection of statistics in a single measurement run.

Currently, we are investigating the precise response of DNA to applied forces and torques at varying salt concentrations, using magnetic torque tweezers (MTT). Preliminary analysis of the torsional stiffness of DNA indicates a weak dependence on salt for forces < 1 pN, where bending deformations play a crucial role, suggesting a coupling between bending and twisting. In contrast, no dependence on salt is observed for higher forces, which suggests that the intrinsic torsional stiffness of DNA does

not depend on ionic strength. At the same time, the bending persistence length is known to decrease with increasing ionic strength, which is in line with the current data on the torsional stiffness of DNA.

Biomolecular Simulation and Computational Biophysics

P49. Coarse-grained Ensemble Modeling of Multi-domain Proteins: Application to Dengue Virus NS5

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An ensemble modeling scheme is implemented to study the flexible biomolecular systems of multi-domain proteins. The method incorporated coarse-grained simulations with experimental small-angle X-ray scattering (SAXS) data and was applied to the DENV2 non-structural protein 5 (NS5). NS5 serves as a key role in viral replication through its methyltransferase (MTase) and RNA-dependent-RNA polymerase (RdRp) domains that are connected by a ten-residue polypeptide segment. The conformation ensemble of low energy agrees well with the experimental SAXS profile. The ensemble construction with the low-energy regularization drives the solution to avoid the arbitrary representation of experimental observables and successfully identified the domain-domain orientation and domain contacting interface of DENV2 NS5. It is found that the absence of energy restraint caused the constructed ensemble of a noisier pattern of conformational space.

P50. DFT calculations for structural analysis of iron-sulfur clusters - impact of model and environment

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Iron-sulfur clusters of the Rieske-type (RC) contain two irons that are linked by two sulfur atoms and are ligated to two cysteines (Cys) and two histidines (His) respectively. They act as active sites of numerous proteins crucial for respiration, photosynthesis and detoxification processes [1]. Density functional theory (DFT)-calculations of RC isolated from the soluble Rieske protein from *Thermus thermophilus* [1] have been performed with different solvents and methods (polarizable continuum model [2] and explicit solvent box) to determine their impact on the structure of the cluster. The isomer shift (δ) which allows derivation of structural properties of the cluster [3] is calculated for each model after geometry optimization. The calculated δ -values are correlated with binding distances of the respective structures.

The DFT-calculations show a clear correlation of bond lengths and δ -values. However, the deviation of calculated and experimental δ -values remains in the range of 0,15 mms⁻¹ for all models. In order to clarify the impact of the functional and basis-sets on the quality of the DFT-calculations, calculations of the Mössbauer parameters of *Pyrococcus abyssi* rubredoxin [5], a single iron ligated to four Cys, with different functionals and basis-sets will be presented.

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P51. Conformational selection and dynamic adaptation upon linker histone binding to the nucleosome

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Linker histones are essential for DNA compaction in chromatin. They bind to nucleosomes in a 1:1

ratio forming chromatosomes. Alternative configurations have been proposed in which the globular domain of the linker histone H5 (gH5) is positioned either on- or off-dyad between the nucleosomal and linker DNAs. However, the dynamic pathways of chromatosome assembly remain elusive. Here, we studied the conformational plasticity of gH5 in unbound and off-dyad nucleosome-bound forms with classical and accelerated molecular dynamics simulations. We find that the unbound gH5 converts between open and closed conformations, preferring the closed form. However, the open gH5 contributes to a more rigid chromatosome and restricts the motion of the nearby linker DNA through hydrophobic interactions with thymidines. Moreover, the closed gH5 opens and reorients in accelerated simulations of the chromatosome. Brownian dynamics simulations of chromatosome assembly, accounting for a range of amplitudes of nucleosome opening and different nucleosome DNA sequences, support the existence of both on- and off-dyad binding modes of gH5 and reveal alternative, sequence and conformation-dependent, chromatosome configurations. Taken together, these findings suggest that the conformational dynamics of linker histones and nucleosomes facilitate alternative chromatosome configurations through an interplay between induced fit and conformational selection. Importantly, these findings reconcile previous experimental and computational studies that revealed a range of different chromatosome configurations.

Reference: Öztürk M. A., Pachov G., Wade R. C., Cojocaru V., 2016, *Nucleic Acids Research*, in press

P52. Probing the structure of the Escherichia coli proteins HdeA and YmgD by pH-titrating MD simulations

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HdeA and YmgD are structurally homologous proteins in the periplasm of Escherichia coli. HdeA has been shown to represent an acid-activated chaperone, which helps E. coli to tolerate very acidic environments, such as the mammalian stomach where the pH varies between 1 and 3. In contrast to the function of HdeA, the function of YmgD has not yet been characterized. We performed pH-titrating molecular dynamics (pHtMD) simulations (1) to investigate the structural changes of both proteins and to assess whether YmgD may also represent an acid-activated chaperone. Upon decreasing pH, HdeA forms partially unfolded dimer structures, which represent a prerequisite for subsequent dissociation. In contrast to the coupled unfolding and dissociation of HdeA, YmgD displays dissociation of the folded subunits, and the subunits do not undergo significant unfolding even at low pH values. The differences in subunit stability between HdeA and YmgD may be explained by the structural features of helix D, which represents the starting point of acid-induced unfolding in HdeA. In summary, the present study suggests that YmgD either is not an acid-activated chaperone or, at least, does not require unfolding for activation.

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P53. Design of ligand interfering with aberrant subcellular processes in Huntington's.

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Huntington's disease (HD) is a fatal and devastating neurodegenerative genetic disorder caused by an expanded CAG repeat in the 5'-end of Huntington gene (HTT). HD penetrance is correlated to the number n of CAG repeats. Mounting evidences emerged that HTT mRNA transcripts with expanded CAG repeats contribute to the pathogenesis, regulate aberrantly several cellular mechanism and bind to proteins in a repeat size-dependent manner to form pathological complexes. One of those involves the Midline-1 protein (MID1) in complex with protein phosphatase 2A (2). Inhibiting the formation of this pathological mRNA/proteins complex, targeting the expanded CAG mRNA transcript, can reduce the HTT overproduction effect (2). Yet, the rational design of molecules specifically targeting expanded

CAG repeats is limited by the lack of structural information. Here, we used well-tempered-metadynamics based free energy calculations to investigate pose and affinity of two ligands targeting CAG repeats for which affinities have been measured (3). Our calculations, consistent with the experimental affinities, uncover the recognition pattern between ligands and the RNA target, providing the molecular basis of their different specificity for CAG repeats (4). By capitalizing on this study, we have identified new compounds with chemical and shape similarity to the highest affinity ligand. RNA pull-down assays show a clear inhibition of the MID1-mRNA CAG complex by one of the new compounds. This work suggests that computational investigations can help design of new molecules targeting RNA with possible beneficial effects for HD.

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P54. Metadynamics simulation of Fibrinogen protomer based on Principal Component Analysis

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Fibrinogen plays a major role in blood clot formation as the precursor of Fibrin. Clot dissolution is controlled by fibrinolysis.

While the biochemical pathways leading to fibrinolysis are largely understood, how these processes take place on a structural level is not.

Previous analysis of Fibrinogen helped to identify and characterize large bending motions occurring at a hinge located in the coiled-coil region of the protein, possibly crucial for the exposure of plasmin cleavage sites during fibrinolysis (1).

Here, the large scale conformational dynamics of Fibrinogen are further analyzed using Metadynamics.

Employing collective variables based on the principal components of motion of Fibrinogen, the full extent of the hinge bending motions is explored revealing a preferred bending direction.

The implications of these findings for Fibrinogen's adsorption properties and function are explored.

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P55. Interpretation of SAXS data using MD simulations

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Small and wide angle X-ray scattering (SAXS/WAXS) are well-established experimental techniques to gain structural information on biomolecules in a solution. SAXS curves are routinely used to extract information on the radius of gyration, aggregation, as well as to construct a low-resolution envelope of the solute.

WAXS has been used to probe the fold of proteins, to detect ligand binding and to characterise the heterogeneous ensembles of both peptides and proteins.

A general method to structurally interpret SWAXS data is, however, still challenging, for two reasons:

(1) Density of the hydration layer differs from the bulk water;

(2) Scattering intensity of the displaced solvent must be subtracted from the scattering intensity of the solute.

All implicit solvent methods require at least two fitting parameters: for the density of the hydration layer and for the excluded solvent. This can lead to loss of information and overfitting.

Therefore, we interpret SAXS data fully based on explicit solvent method. This method provides both accurate description of the hydration layer and the excluded solvent, at the price of a higher computational cost. No solvent-related fitting parameters are required.

Here, we tried to predict SAXS curve of n-decyl- β -D-maltoside, n-dodecyl- β -D-maltoside, n-dodecylphosphocholine micelles using MD simulations. Also, we investigated counter ion cloud

impact on SAXS curves calculation of two charged proteins: glucose-isomerase and bovine serum albumin (BSA).

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P56. Molecular dynamics simulations on different fibrillar A-beta(40) species with three-fold symmetry

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Alzheimer's Disease (AD) is the most prevalent neurodegenerative disorder and the main cause for dementia in industrial nations. One hallmark of AD is the development of senile plaque deposits in the brain that consist primarily of fibrillar amyloid beta (A-beta) peptides. A-beta is a short peptide comprising

40 to 42 residues, but nevertheless exhibits a vast conformational variability and a plethora of oligomeric states, which makes experimental studies about its structure and aggregation rather challenging.

Recently, Lu et al. published a solid state NMR structure of an A-beta(1-40) fibril isolated from an AD patient (PDB code 2M4J)(1). The structure shows three-fold symmetry around the fibril axis with a central water channel and is thus markedly different from A-beta(1-42) fibril structures. Previously, we have investigated the stability of fibrillar A-beta(42) oligomers of different size by means of molecular dynamics (MD) simulations leading to a model for longitudinal and lateral fibril growth.(2,3)

Here, we present all-atom MD simulations in explicit water based on the patient-derived A-beta(40) fibril to elucidate how its conformational stability depends on the oligomer size. An infinite A-beta fibril was investigated as well to study the boundary effects of the finite oligomers.

Moreover, it is known from experiment that several A-beta species of different N-terminal length exist in vivo affecting the peptide's aggregation behaviour. We thus investigated the influence of the first eight A-beta residues upon the structure and dynamics of the fibrillar oligomers and the infinite fibril of A-beta(40).

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P57. Interactions between PEG and Proteins Investigated Using Molecular Dynamics Simulations

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Polyethylene glycol (PEG) is a polymer with a vast range of applications, including medical and biochemical applications. PEG is often used to improve the therapeutic efficacy of drugs, proteins, liposomes or nanoparticles through the PEGylation process, which helps reducing unspecific interactions with the biological fluids of the host organism. Notwithstanding this fact, PEG is not totally inert. Proteins do adsorb on the surface of PEGylated nanoparticles and immune reaction to PEG are also known. Here we use molecular dynamics simulations to investigate the non-covalent interactions taking place between PEG and several blood proteins. The simulations are used to measure the preferential binding coefficient of PEG for proteins, and reveal recurring patterns of interaction involving specific aminoacids. The latter could be used for the development of coarse grained representations of protein-PEG interactions and may provide the basis for understanding the properties of protein coronas formed around PEGylated nanoparticles.

P58. PMF calculations of solute permeation across UT-B and AQP1: A comparison between MD and 3D-RISM

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Membrane channels facilitate the efficient and selective flux of various solutes across biological membranes. A common approach to investigate the selectivity of a channel has been the calculation of potentials of mean force (PMFs) for solute permeation across the pore. PMFs have been frequently computed from molecular dynamics (MD) simulations, yet the three-dimensional reference interaction site model (3D-RISM) has been suggested as a computationally efficient alternative to MD.

Whether the two methods yield comparable PMFs for solute permeation has remained unclear.

In this study, we calculated potentials of mean force for water, ammonia, urea, molecular oxygen, and methanol across the urea transporter B (UT-B) and aquaporin-1 (AQP1), using 3D-RISM, as well as MD simulations and umbrella sampling. For PMFs of water permeation we found reasonable agreement between the two methods, with differences of < 3 kJ/mol. In contrast, we found stark discrepancies for the PMFs for all other solutes. Comparison of the PMFs suggests that 3D-RISM may underestimate effects from hydrophobic solute-channel interactions, thereby, for instance, missing the urea binding sites in UT-B. Furthermore, the orientational averages inherent to 3D-RISM might lead to discrepancies in the narrow channel lumen. These findings suggest that current 3D-RISM solvers provide reasonable estimates for the PMF for water permeation, but that they are not suitable to study the selectivity of membrane channels with respect to uncharged non-water solutes.

P59. Simulating force response at cell junctions: desmoplakin as a molecular force sensor?

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Desmosomes are cell-cell junctions with a key role in mechano-sensation. However, it is currently unknown how the desmosomal complex and its proteins respond to mechanical force, transmitting chemical signals. We study desmoplakin, one of the obligate desmosomal proteins, identifying its possible role in mechanosensing. The most striking feature of this protein is the presence of an SH3 domain embedded in one of its six spectrin repeat domains. Intriguingly, the spectrin repeats hide the typical SH3 binding site, suggesting that either (i) the SH3 domain serves as a stabilizer of the protein through its interaction with one of the spectrin repeat, or (ii) it is activated only under force, initiating a signaling cascade for mechanosensing.

To explore these alternatives, we performed equilibrium as well as force-probe Molecular Dynamics simulations on the wild-type and relevant mutants. To bridge towards physiologically relevant time scales, we repeated our simulations through a wide range of pulling velocities with a total simulation time of several tens of microseconds.

We find desmoplakin to feature a clear sawtooth-like force profile, with each peak corresponding to modular spectrin repeat unfolding events followed by the final SH3 unfolding. The SH3 domain stabilizes desmoplakin, and is unlocked by force to interact with binding partners. We also simulate known disease mutants and find altered mechanical response, suggesting their direct impact on DP mechano-sensing. Interestingly, the homologous cytosolic plectin protein features a similar unfolding pathway, but at significantly lower forces. Our results suggest a direct role of SH3-spectrin repeat proteins in cellular mechanotransduction, and will be validated by direct comparison to AFM measurements carried out by our collaborators.

P60. Stratum Corneum Structure – Insight from Molecular Simulations of Bilayers and Multilayers

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Stratum corneum, the uppermost layer of the skin, forms a highly impermeable barrier on the surface

of our bodies. Though an extensive experimental effort was focused on this tiny layer, its molecular structure is still not precisely known. The composition of stratum corneum includes various kinds of ceramides, free fatty acids with long saturated tails, cholesterol and cholesterol sulphate. With electron microscopy and other techniques a short and long periodicity phase was observed, however the molecular arrangement of individual lipids is still under debate. The studies of the structure of stratum corneum are challenging both by experimental and theoretical tools.

We used molecular dynamics (MD) simulations in order to investigate the stability and structure of ceramide layers. (1) We built pure ceramide bilayers in various environments and analyzed the role of water molecules on the stabilization of ceramide layers. Further, we built bilayers composed of ceramides, free fatty acids and cholesterol and by a simulated annealing we analyzed the interactions between individual lipids and their clustering behavior. And finally, we created double bilayers and in various temperatures we monitored the behavior of water molecules that penetrated through one of the bilayers in order to reach the head group region. We also analyzed the exchanges of ceramide conformations from initial hairpin to extended. And we also monitored the flip-flops of cholesterol and free fatty acids, the changes in their concentrations in various regions during the simulations. The cholesterol and free fatty acids were able to exchange fast in between the membrane leaflets. We believe that these fascinating movements of lipid molecules are able to provide a high-resolution view into the possible molecular structure of stratum corneum.

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P61. Computational metallomics of the anticancer drug cisplatin

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Cisplatin, cis-diamminedichlorido-platinum(II), is an important therapeutic tool in the struggle against different tumors, yet it is plagued with the emergence of resistance mechanisms after repeated administrations. This hampers greatly its efficacy. Overcoming resistance problems requires first and foremost an integrated and systematic understanding of the structural determinants and molecular recognition processes involving the drug and its cellular targets.

Using hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) simulations, validated by spectroscopic experiments (including NMR, and CD), we have worked out for the first time at atomic level the structural determinants in solution of platinated cellular substrates. These include the copper homeostasis proteins Ctr1, Atox1, and ATP7A (1-3). All of these proteins have been suggested to influence the pre-target resistance mechanisms. Furthermore, coupling hybrid QM/MM simulations with classical Molecular Dynamics (MD) and free energy calculations, we have characterized the structural modifications and the free energy landscape associated with the recognition between platinated DNA and the protein HMGB1, belonging to the chromosomal high-mobility group proteins HMGB that inhibit the repair of platinated DNA (1). This may alleviate issues relative to on-target resistance process. The elucidation of the mechanisms by which tumors are sensitive or refractory to cisplatin may lead to the discovery of prognostic biomarkers. The approach presented here could be straightforwardly extended to other metal-based drugs.

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P62. Cytochrome P450 Reductase Simulation: Conformation changes and Cytochrome P450 complex

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The Cytochrome P450 Reductase (CPR) is large 680 amino acids long microsomal multidomain enzyme responsible for electron donation to its redox partner cytochrome P450 (CYP) involved in drug metabolism. Electron transfer (ET) chain is mediated by two riboflavin-based cofactors – flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) within their respective domains and nicotinamide adenine dinucleotide phosphate (NADPH). During this electron transfer CPR undergoes several structural changes in open and closed state with domains in different degree of contact. In spite of the fact that CYP-CPR complexes play a key role in drug metabolism, the atomistic mechanism of structural rearrangements during complex electron transfers is still lacking.

Here, we present the results of our study on structural changes during CPR multidomain complex movement between individual electron transfers using classical molecular dynamics (MD) and metadynamics (MTD) simulations with cofactors of NADPH, FAD and FMN in resting state. Homology model of human CYPOR in both conformations (open and closed) were embedded into pure dioleoylphosphatidylcholine (DOPC) bilayer. After systems equilibration structural changes of protein, anchor and cofactor movement were studied. We were able to select possible CPR-membrane orientation which would allow interaction with cytochrome P450. In addition, MTD simulations describing closing mechanism were performed pointing out for so called to be the most flexible part during conformation changes. At least, we successfully created model of CPR with its redox partner cytochrome P450 3A4 both embedded in membrane. CPR-CYP model was used for prediction of amino acid residues responsible for interprotein electron transfer.

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P63. Accelerated molecular dynamics study of the effects of surface hydrophilicity on protein adsorption

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The adsorption of streptavidin is studied on two surfaces – graphite and titanium dioxide – using accelerated molecular dynamics. Adsorption on graphite leads to strong conformational changes while the protein spreads out over the surface. Interestingly, also the adsorption on the highly hydrophilic rutile surface induces a considerable spreading of the protein. We pin down the cause for this unfolding to the interaction of the protein with the ordered water layers above the rutile surface. For special orientations, the protein penetrates the ordered water layers and comes into direct contact with the surface where the positively charged amino acids settle in places adjacent to the negatively charged top surface atom layer of rutile. We conclude that for both surface materials studied, streptavidin changes its conformation so strongly that it loses its potential for binding biotin. Our results are in good qualitative agreement with available experimental studies.

P64. 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. Furthermore, optimal proton transfer pathways are determined from explicitly or semi-explicitly (water positions from MD simulations) sampled Transition Networks. In addition to the model calculations we investigated the proton transfer within CcO's D-channel, thereby elucidating its steric and hydrational gating.

P65. Protonation state dependent Communication in Cytochrome c Oxidase

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Proton transfer in Cytochrome c oxidase (CcO) from the cellular inside to the binuclear redox center (BNC) can occur through two distinct pathways, the D- and K-channel. In order for the protein to function as both, redox enzyme as well as proton pump, proton transfer out of either of the channels towards the BNC or into the protein towards a protein loading site, and ultimately through the membrane, must be highly regulated. The O→E intermediate of CcO is the first redox state in its catalytic cycle where proton transfer through the K-channel, from K362 to Y288 at the BNC, is important. Molecular dynamics simulations of this intermediate with sixteen different combinations of protonation states of key residues in the D- and K-channel show the mutual impact of the two proton conducting channels to be protonation state dependent. Both, strength as well as means of communication, correlations in positions or along the hydrogen-bonded network, change with the protonation state of the K-channel residue K362. The conformational and hydrogen bond dynamics of the D-channel residue N139 is regulated by an interplay of protonation in the D-channel and K362. N139 thus assumes a gating function by which proton passage through the D-channel towards E286 is likely facilitated for states with protonated K362 and unprotonated E286, which would in principle allow proton transfer to the BNC, but no proton pumping until a proton has reached E286.

P66. Flexibility of a wave-inducing genetic cascade explains evolutionary diversity in insect early embryo

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While the basic body plan of animals is highly conserved, earlier and later embryonic events are quite diverse, a phenomenon termed 'the hourglass model'. All insects have a segmented body plan, where anterior segments arise in a non-growing 'blastoderm', and posterior segments arise in a growing 'germband'. However, insects differ widely in the number of blastodermal versus germband segments. Here we show, using in vivo and in silico evidence, that patterning in both blastoderm and germband of the beetle *Tribolium* is based on the same flexible mechanism: a genetic cascade of gap genes that induces waves of gene expression. The mechanism is capable of converting blastodermal to germband fates and vice versa. Using RNAi and heat shock perturbations, the mechanism was dissected and blastodermal fates were induced in the germband, and germband fates were induced in a blastoderm-like morphology. Computational modeling suggests two genetic programs are involved, in line with the recent finding of non-redundant shadow enhancers in *Drosophila*. Our work suggests a simple mechanism for evolutionary flexibility in early insect development.

P67. Hierarchical organization and resilience of biological networks

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Living systems appear to epitomize resilient behavior: they succeed at maintaining certain minimal functionality in spite of permanent perturbations, environmental shifts and accumulated damage. It is often suggested that the concept of network resilience may help provide a unified framework to model such an exceptional resilience: if the network structure used to model a certain biosystem proves resilient, then this may be the foundation of the ability of the system to maintain activity and functionality in the presence of damage. We will present a numerical and theoretical study of the resilience of hierarchical modular networks (HMNs), relevant in the description of biological systems, and show how our results carry over to real biological networks, such as the mapping of structural connectivity of the human brain, or human connectome. In particular, we emphasize the role of

hierarchical ordering in reinforcing the network structure against failures. We provide a detailed numerical analysis of spreading and percolation phenomena in HMNs, showing the emergence of extended scaling windows as a result of the hierarchical order. These results are reminiscent of our previous results on rare region effects in brain networks [1–3], suggesting that HMNs are able to confine activity and damage into localized regions, avoiding macroscopic spreading. By using concepts of the canonical theory of perturbations, we show the spectral fingerprints of enhanced resilience of HMNs, as they emerge from the study of the graph Laplacian. This result enables us to highlight the parameters that tune HMN resilience, paving the way for further studies of network controllability.

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P68. Molecular dynamics free-energy calculations on VDAC helix unbinding

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The voltage-dependent anion channel (VDAC) is located in the outer mitochondrial membrane (OMM) and can be present in a closed or open configuration, depending on whether the N-terminal α -helix occupies the central pore or protrudes into the cytoplasmic region. The latter state is known

to be promoted by the presence of another protein resident in the OMM (Bcl-xL) [1] to whose cytoplasmic domain the helix may bind [2]. The goal of this study is to quantify the free-energy contributions associated with (i) releasing the helix from the pore (in practice complicated by the relatively unstable nature of the helix structure) and (ii) its noncovalent binding to Bcl-xL (in practice complicated by the disordered nature of the ligand in solvent) using molecular dynamics simulation.

The work shown here is focused on the first step. Using a Hamiltonian replica exchange sampling scheme, we calculate a potential of mean force along the z-coordinate of the helix-pore center-of-mass distance. The raw free energy required to translocate the helix to a position immediately outside the pore is on the order of a few tens of kJ/mol. The pathway exhibits multiple minima for which we characterize important helix-pore interactions. Although some minima appear to be separated by low free energy barriers, a 60 ns simulation of VDAC starting from the x-ray structure only presented the helix close to its native position.

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P69. Large Scale Validation of GROMOS Force Fields for Biomolecular Simulations

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Molecular dynamics (MD) simulations of biomolecules offer an insight into the relationship of protein structure, dynamics, and function at the atomic level.

The fundamental building block of a MD simulation is the force field - it describes the interatomic interactions and consists of a set of empirically derived parameters. It is developed to match a number of experimental results as closely as possible to increase the reliability of a simulation conducted with this force field (1).

The GROMOS force fields are parametrised to match a set of thermodynamic properties of small molecules as well as spectroscopic properties of biomolecules, such as torsion angle distributions and NOE violations as collected from NMR experiments.

The validation of the previous GROMOS force fields included the thorough investigation of a few proteins and peptides, hence lacking a solid statistical basis for their validation. We have compiled a set of 60 proteins, 5 fast-folding peptides, and 3 β -peptides to alleviate this problem.

With the analyses conducted so far, we can show that the most recent GROMOS force fields (54A7 (4) and 54A8 (5)) are superior to their predecessors (45A3 (2), 53A6 (3)) in terms of protein stability and dihedral angle distributions. The dihedral angle analysis also shows which amino acid backbone dihedrals are a promising target for further force field improvement.

For the first time, the four latest major GROMOS force field versions have been compared on a wide range of proteins and peptides with the same post-simulation analyses. This large set of trajectories and analysis data could be a valuable resource and benchmark for future force field development.

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P70. A β protofilament propagation – studied by atomistic Molecular Dynamics Simulation.

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The incurable Alzheimer's disease is associated with fibrous plaques deposits of A β -amyloid peptides in brain tissue [1]. Detailed insight into fibril growth and putative neurotoxic intermediates like oligomers and protofilaments has largely remained elusive, although this is of outstanding relevance for drug development [1,2]. As experimental access to the highly dynamic growth intermediates is very limited, Molecular Dynamics (MD) simulation serves as a powerful complementary tool due to both its atomic spatial and femtosecond time resolution [3].

In this work, atomistic Molecular Dynamics simulations in explicit solvent are used to study A β protofilament propagation by the binding of an additional monomer. Extensive Umbrella Sampling simulations in the microsecond range provide insight into the free energy profile of association, conformational transitions and the key role of hydrophobic solvent expulsion during the dock-lock binding mechanism [4]. Furthermore, the development of an Enhanced Sampling protocol for A β binding free energy calculation allows the accurate and efficient quantification of biomedically relevant parameters related to A β filament growth, including the comparison between A β 40 and the more toxic isoform A β 42 as well as pathogenic mutations and elongation inhibitors.

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P71. Studying the DNA Damage Recognition Mechanism of XPA employing Molecular Dynamics Simulations

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Nucleotide excision repair (NER) is a DNA repair system exhibiting remarkable substrate promiscuity. As a consequence, defects in the NER system are responsible for a variety of diseases including Xeroderma Pigmentosum and the Cockayne Syndrome [1].

More than 30 proteins are involved in NER, one participant of major significance is the XPA protein, which is of central importance for the initial damage recognition [2]. Previous experimental investigations have shown that XPA does not recognize the lesion itself, but instead senses specific alterations in the mechanics of the DNA [3]. The known crystal structures in complex with DNA suggest that the bending flexibility is paramount to whether the substrate is bound by XPA or not [3]. Molecular Dynamics (MD) simulations are an ideal tool to capture the dynamics of the DNA-protein complex at the atomic level and at femtosecond resolution. In this study, several XPA-DNA complexes and the unbound DNA molecules have been studied comparatively with extensive MD simulations. The simulations indicate that the structure of the damaged DNA is already in the absence of XPA closer to the bound form than regular DNA. Recording differences in the mechanical responses of the DNA substrates provides further insight into the recognition mechanism of the XPA protein.

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P72. Membrane Phase Transition during Heating and Cooling: Molecular Insight into Reversible Melting

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Bacteria change their lipid composition dependent on the growth temperature so that the membrane keeps its optimal properties. With increasing temperature, lipid bilayers undergo an isothermal gel-fluid phase transition, which plays an essential role in many physiological phenomena.

In the present work, first-order phase transition under variable heating and cooling rates are investigated for the dipalmitoyl phosphatidylcholine (DPPC) lipid bilayer by means of atomistic molecular dynamics simulations. We quantitatively compare alternative methods to track the melting temperature T_m . The resulting T_m is seen to be independent of the heating rate for low heating rates (0.05 - 0.3 K/ns) implying reversible melting, and increases for increasing heating rates (0.3 - 4 K/ns) or decreasing cooling rates (2 - 0.167 K/ns). The reported dependency of the melting temperature on the heating rate is in perfect agreement with a two-state kinetic rate model (1). For reversible melting, expansion and shrinkage of melting seeds displaced from each other between the membrane layers was observed.

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P73. Roles of fusion peptides in membrane fusion

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Efficient and controllable fusion of biological membranes is known to be driven by a cooperative action of fusion peptides. Both SNARE proteins(1) and their analogs, peptides E and K(2), are known to form a coiled coil complex thus overcoming the electrostatic repulsion between the membranes and bringing them into close proximity. Less is known about the role of fusion peptides in the subsequent steps of the fusion process.

Here, we show that peptide K has a two-fold function in membrane fusion. Next to the coiled-coil complex formation with peptide E, it is observed to bind to the membrane and to alter its properties. Namely, adsorbed peptides K induce a positive membrane curvature and enhance lipid protrusion. These effects were suggested to be crucial for the formation of the first hydrophobic contact between the bilayers (3,4). K moreover sorts phosphatidylethanolamine to its vicinity and thus to the area of positive curvature, therefore possibly fostering membrane stalk formation.

Interestingly, the primary sequence of the transmembrane domain (TMD) of the SNARE proteins has been shown to determine the speed and the extend of membrane fusion(5). The speed of membrane fusion is known to depend on the number of SNARE complexes formed. Therefore, we have investigated the oligomerization propensity of the TMDs of the wild type synaptobrevin and its mutants as well as the effects of the primary sequence on membrane properties and on the secondary structure and flexibility of the juxtamembrane domain. Our results hint to distinct roles of the fusion peptides at different stages of membrane fusion.

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P74. Quaternion-based calculation of rotational diffusion in MD

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Rotational diffusion is a fundamental property of biomolecules that describe its tumbling in solution as influenced by molecular shape and solvent viscosity. It is of utility in the study of disorder and molecular complexation events, where knowledge the molecule's tumbling behaviour assists in the identification of the correct molecular structure and stoichiometry. Therefore, NMR relaxation and polarised fluorescence techniques are often applied to measure experimental tumbling, yielding components of the rotational diffusion tensor, D_{rot} .

Prediction of D_{rot} from all-atom simulations (MD) is currently limited by two issues: (1) the viscosity of popular water models, which overpredict D_{rot} by varying margins, and (2) the length of trajectories required to converge angular autocorrelation functions involved in the calculation.

In response to this, we examined the ability of a coarse-grained approach to resolve both issues. A quaternion-based approach was derived from the broader literature, which yields D_{rot} without recourse to atomic vectors not necessarily available. Tests of this approach in 12 systems ranging 5 to 150 kDa, using the MARTINI forcefield, results in prediction of isotropic D_{rot} to within 20% for proteins up to ovalbumin in size, and within 10% if proteins with significant disorder was excluded. This accuracy decreases somewhat for anisotropic components, which are adversely affected by small deviations of protein shape in MARTINI, as well as its inability to accurately model disordered coils as-is.

We compare this to reference all-atom simulations, which do show accurate prediction of anisotropy. Thus, a multiscale-approach may be optimal for the prediction of rotational diffusion as well as NMR relaxation from simulated dynamics alone.

P75. Protein-Induced Membrane Curvature

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The (local) curvature of cellular membranes can function as a sorting mechanism for transmembrane proteins, e.g. by accumulation in regions of matching spontaneous curvature, as shown recently for potassium channel KvAP and water-pore AQP0 [1]. However, the direction of the reported spontaneous curvature levels as well as the molecular background could not be addressed experimentally yet.

Using coarse-grained and atomistic molecular dynamics (MD) simulations, we find matching levels of spontaneously induced curvature for the homologous potassium channel Kv 1.2/2.1 Chimera (KvChim) and AQP0 when embedded in unrestrained, finite bilayers of POPC lipids. Furthermore, additional information about the direction of curvature can be accessed directly from our simulations. Results obtained from coarse-grained systems are in excellent agreement with the experiments [1] with values of -0.036 nm^{-1} and 0.019 nm^{-1} for the spontaneous membrane curvature induced by KvChim and AQP0, respectively.

Atomistic simulations of both systems show spontaneous curvatures comparable to their coarse-grained counterparts, and allow for detailed investigation of its origin, especially in terms of protein-lipid interactions. Here, uneven distribution and organization of POPC lipids at the interface of KvChim establish basal negative curvature, which is further modified by the dynamics of the protein itself.

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P76. Synaptobrevin - Peptide Induced Membrane Curvature

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Nerve signal transduction among nerve cells is accomplished via neurotransmitter release upon rapid fusion of synaptic vesicles with the plasma membrane. This calcium-triggered process is known to be mediated by members of the SNARE protein family, which assemble into the so-called SNARE complex during fusion. Recent studies show that in presence of membrane curvature the activation energy to overcome upon first contact of the fusing membranes is significantly decreased [1,2]. Here, by means of coarse-grained molecular dynamics simulations we elucidate, whether one of the multiple roles of synaptobrevin, which is a SNARE protein anchored to the synaptic vesicle, is to induce local

membrane curvature similarly to viral fusion peptides. At first, the role of the positively charged juxtamembrane domain (JMD) on the induction of membrane curvature is studied, and then, the specificity of the transmembrane domain (TMD) is revealed. To this end, two different TMD mutants are introduced, which are known to significantly influence the extent and speed of membrane fusion [3].

Additionally, the importance of lipid composition for protein-induced membrane curvature is illustrated by investigating membranes including cholesterol, negatively charged lipids or lipids spontaneously inducing negative curvature.

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P77. Protein and RNA structure prediction through co-evolutionary information

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Exploring the interrelationship of structure and function is crucial for the understanding of molecular life. Despite significant progress of experimental methods, the structural characterization of important structures for both proteins and RNA remains challenging. In recent years, increasingly ubiquitous availability of sequential information and advanced statistical analysis has allowed to trace the co-evolution of residues and predict partial contact maps. Direct-Coupling Analysis (DCA) [1] develops a global model mimicking the evolutionary fitness landscapes of protein families [2] by quantifying the coevolution of residue pairs. Such information has proven useful in the prediction of tertiary protein structure, conformational transitions[3], protein complexes[4], membrane proteins, homooligomers [unpublished data] and similarly RNA structures[5] with several blind predictions later confirmed by experiments. Moving beyond the structural implications, DCA can infer mutational landscapes and capture epistatic couplings [2]. For RNA, it is comparably simple to predict secondary structure. Predicting tertiary contacts has remained an elusive task met with limited success. In contrast, our novel maDCA is able to extract tertiary contacts from genomic data. We further demonstrate that these tertiary contacts are sufficient to systematically improve tertiary RNA prediction quality [5]. Considering the large gap of known ncRNA sequences to experimentally resolved tertiary structures, we are convinced that this will significantly impact all structural RNA related research.

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P78. Virus therapy of glioblastomas

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Clinical research has shown that Vesicular Stomatitis Virus (VSV) has strong oncolytic properties. In parallel, biophysical models of virus treatment of tumors have been developed. However, experiments have been never compared quantitatively with computational models. We describe a new spatial model for VSV-glioblastoma interaction with time-delay effects. For the virus dynamics, we obtain results consistent with the in vitro experimental speed data. The tumor speed is given by the minimum of a very simple function that nonetheless yields results within the experimental measured range. We show that the delay time is the crucial parameter in this kind of models. Our new model can satisfactorily predict the front speed for the lytic action of oncolytic VSV on glioblastoma observed in vitro. We provide a basis that can be applied in the near future to realistically simulate in vivo virus treatments of several cancers.

P79. A Molecular Modelling Approach to the Delineation of Oxidative Stress in Alzheimer's Disease.

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Oxidative stress is highly prevalent in the Alzheimer's disease (AD) brain but its precise role in AD pathology is not well-understood. Free radicals can oxidise the lipids that form the plasma membrane, a result which has shown to promote Ab-membrane interactions. Free radicals can also abstract hydrogen from the Ca of Gly and Ala residues, which initiates the a to b unfolding of oxidised model peptides [1,2]. Ab undergoes a similar conformational change, which may be related to the formation of toxic Ab oligomers. Moreover, it has been proposed that A β -membrane interactions subsequently disrupts neuronal Ca²⁺ homeostasis [3].

In an effort to delineate the role of Ab and lipid oxidation in AD pathology, we monitored the changes to the structure of oxidised Ab peptides in different solvents, and a bilayer comprised of oxidised 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) and cholesterol using molecular dynamics (MD) simulations. An oxidised Gly25 caused Ab to form b-sheets in a solvent-dependent manner, whereas oxidised POPC affected the membrane curvature, bilayer thickness, and the area per lipid in a concentration-dependent manner. These studies are an attempt to delineate the role of oxidative stress in the pathology of AD as it relates to Ab conformational change and Ab-membrane interactions.

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Biospectroscopy, EPR, and NMR

P80. EPR- and IRRAS studies on alpha-Synuclein

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α -Synuclein is a protein composed of 140 amino acids that forms a major constituent of the Lewy bodies characteristic of Parkinson's disease. Although no precise function has yet been determined, binding to membranes seems to be important for its physiological role. α -Synuclein lacks a well-defined secondary structure in free solution, but adopts a more ordered structure upon binding to negatively charged membranes.

In order to study the membrane-bound state of α -Synuclein, single cysteine mutants labelled with the MTS spin label are examined by studying the interaction between α -Synuclein and liposomes composed of the negatively charged lipid POPG. CW-EPR spectroscopy is used to obtain information about the dynamics and polarity of the environment of the incorporated spin label. The DEER-technique is used to obtain information about the self-assembly on membranes by nanoscale distance measurements. Further examinations of the α -Synuclein wild-type include the Infrared-Reflection-Absorption-Spectroscopy (IRRAS) in order to study the interaction of proteins with lipid monolayers and to study changes of secondary structure upon self-assembly of proteins (1).

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P81. Electronic and vibrational properties of the NO heme interactions of Nitrophorin 2

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The nitrophorins (NPs) comprise an unusual family of proteins which form stable ferric heme nitric oxide (Fe(III)-NO) complexes for a long period of time. They are found in the salivary glands of the blood-sucking kissing bug *Rhodnius prolixus*, where the insects use the NPs to store the highly reactive signal molecule NO and transport it to the victim's tissues. The released NO effects in vasodilatation and reduces blood coagulation, what facilitates the blood meal of the insect.

Nuclear Inelastic Scattering (NIS) data sets of ⁵⁷Fe enriched NP2-NO and the mutant NP2-NO L132V show a strong band around 600 cm⁻¹ which is due to modes with significant Fe-NO motions [1,2]. Combined quantum mechanical and molecular mechanical (QM/MM) calculations of the whole proteins indicate a considerable influence of the protonation states of the heme propionates not only on the vibrational properties of the Fe-NO entities, but also on the electronic ground states of the NO heme adducts.

This work has been supported by the research initiative NANOKAT and by the German Federal Ministry of Education and Research under 05K13UK2.

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P82. Photophysical characterization of novel pyridinium azobenzenes

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Azobenzenes are light sensitive molecules widely used to control molecular motions. When exposed to light of a particular wavelength the molecules switch from the trans form to the cis form (1). Over

time, the molecules relax back into the thermodynamically stable trans state. This back relaxation happens on the time scale of hours and even days for non-substituted azobenzene molecules, whereas substituted molecules may relax within seconds or even nanoseconds (2, 3). Nowadays, efforts are concentrated on synthesizing water-soluble and biocompatible azobenzene probes with absorption bands in the red region (4, 5).

Here, we have synthesized and characterized water-soluble and fast switching azo compounds. Their short lived cis forms with half-life times in the microsecond to millisecond time range could only be studied with nanosecond flash photolysis. We investigated the effect of cyclic and acyclic substituents as well as the bromine atom on the half-life of the cis form in phosphate buffered saline (PBS) and in a solvent with higher viscosity. Moreover, we have observed irreversible photoconversion of disubstituted azo compounds. The photoproducts featured reversible isomerization. These disubstituted azo compounds also showed pH-dependent isomerization kinetics.

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P83. Advanced EPR spectroscopy sheds light on the radical transfer in E. coli ribonucleotide reductase

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Ribonucleotide reductases (RNRs) are essential in life because they catalyze the synthesis of DNA building blocks in all living organisms. The E. coli RNR uses a catalytic cycle that involves proton-coupled electron transfer (PCET) from a tyrosyl radical (Y122•) in subunit β to a cysteine (C439) in the active site of subunit α , which subsequently initiates nucleotide reduction. This oxidation occurs over 35 Å and involves a specific pathway of redox active amino acids present both in α and β subunits (1). The mechanisms of PCET steps at the interface of the $\alpha\beta_2$ complex remain puzzling due to lack of structural information in this region. Two strategies are developed to investigate the nature of PCET at the subunit interface: (i) the essential radical transfer pathway tyrosine 731 is trapped during forward PCET before the catalysis via incorporation of 3-aminotyrosine (NH₂Y731- α); (ii) the adjacent pathway residue 356 is trapped during reverse PCET after the catalysis via incorporation of (2,3,5)F3Y at position 122. Once these naturally transient radicals are trapped, their structure and hydrogen-bonding environment are studied by advanced EPR spectroscopy combined with rapid-freeze quench (RFQ) methodology and DFT calculations. Our multi-frequency EPR, DFT, ENDOR and DEER data on 3-aminotyrosyl radical (NH₂Y731•- α) indicate a large conformational change of this pathway residue (2). Furthermore, trapped Y356• is studied via RFQ- and 263 GHz EPR spectroscopies. Due to utility of these state-of-the-art techniques, we, for the first time, could demonstrate the interaction between the two essential pathway tyrosines 731 and 356 over the subunit interface. The conformational change of residue 731 might be the key to drive the radical transfer chemistry at the subunit interface and also to its interaction with adjacent essential pathway residue 356. (1) U. Uhlin and H. Eklund, 1994, *Nature*, 370, 533–539. (2) M. Kasanmascheff et. al., 2016, *Chemical Science*, 7, 2170-2178.

P84. Membrane interaction and aggregation of α -synuclein monitored by ATR-FTIR difference spectroscopy

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ATR-FTIR difference spectroscopy facilitates high surface sensitivity by canceling the background absorbance signal. We use this technique to study the aggregation of α -Synuclein. We also use ATR-FTIR difference spectroscopy and solid supported lipid bilayers (SSLB) to follow the conformational changes of α -Synuclein in interaction with membrane. The wildtype α -Synuclein and the deletion mutant exon 3 were studied. We clearly observe that in absence of membrane, the wildtype α -Synuclein aggregates after several hours of incubation at room temperature, while the deletion mutant exon 3 does not perform any conformational change. However, although α -Synuclein is intrinsically disordered in solution, upon membrane interaction an increased fraction of α -helical structure is observed for both the wildtype protein and the deletion mutant exon 3. Moreover, we report initiation of an aggregated protein structure that grows immensely within several minutes of interaction with membrane, for the wildtype α -Synuclein as well as the deletion mutant exon 3. This data highlights the importance of membrane interactions in α -Synuclein aggregation.

P85. Conformational changes of mVDAC1 upon tBid binding studied by pulse EPR

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Voltage-dependent anion-selective channels (VDACs), also known as mitochondrial porins, are the major proteins of the outer mitochondrial membrane of eukaryotes. They form membrane-spanning β -barrels and act as general diffusion pores for small hydrophilic molecules, adopting an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV. VDACs facilitate the exchange of ions and molecules between mitochondria and the cytosol and are regulated by the interactions with other proteins and small molecules.(1) Furthermore, it is known that VDACs are conserved mitochondrial elements of the apoptosis pathway in both plant and animal cells.(2)

The high resolution structures of human³ and murine⁴ VDAC1 (h/mVDAC1) revealed a 19-stranded β -barrel with an α -helix located in the middle of the pore which is thought to be the key element for regulating the conductance of ions and metabolites through the channel.

We applied site directed spin labeling and distance measurements by double electron-electron resonance (DEER) spectroscopy to study the influence of tBid (truncated Bid), an activated BH3-only pro-apoptotic member of the Bcl-2 protein family, on mVDAC1. The results from pulse EPR spectroscopy are compared to simulated distance distributions obtained from molecular dynamics simulations of mVDAC1 in a lipid bilayer. We observed a clear influence of tBid binding on the inter spin distance distributions obtained for spin labeled mVDAC1, suggesting that interaction with tBid "closes" the anion channel by fixing the N-terminal α -helix within the pore.

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Photobiophysics and Biological Electron and Proton Transfer

P86. Structural Relaxation of DNA upon Electron Transfer and De-Excitation

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Upon irradiation, deoxyribonucleic acid (DNA) undergoes number of processes, which may lead to a DNA damage. These changes have often profound consequences to DNA's biological function resulting in erroneous cellular states eventually (1). DNA nucleobases can be oxidized forming a positive hole that can travel along the DNA strand (2). It seems that no structural information exists about this process. In particular it is unknown, what does the DNA structural response on electronic changes look like, and how quick such a response is.

To this point we have performed all-atom molecular dynamics (MD) simulations equivalent to 50 μ s of trajectories in both equilibrium and non-equilibrium regimes. Two events taking place in the central TA step of the d(CGTACG)₂ hexamer have been studied: i) the transfer of electron between the two adenines, and ii) the de-excitation of an adenine. The DNA structure was described in terms of helical parameters in the standard reference frame (3).

According to the simulations, the most dynamic coarse-grained coordinate is the shift of the TA step, which corresponds to the relative movement of the base pairs in the direction from/to major/minor groove. Most of the structural parameters show a strong fluctuation within the first picosecond. We have identified four characteristic times of the subsequent structural decay. The quickest below 1 ps is modulated by nearby water molecules and is more pronounced after the de-excitation than after the electron transfer. The slowest one reaches 1 ns and may originate in the DNA itself. There seems to be no fundamental difference between de-excitation and electron transfer in terms of relaxation times. However, the magnitude of structural as well as energetic changes in the former case is much lower than in the latter one.

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P87. Photoinduced processes of free bilins in solution: fs TA absorption spectroscopy at phycocyanobilin

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Bilins are linear tetrapyrrols with rich and divers photochemistry in solution (1), involving C-C single- and double-bond isomerization of one or several of the pyrrole methine bridges. When bound as cofactor to plant (or bacterial) phytochrome proteins they serve as chromophores for a photoinduced signal cascade enabling red-light sensing and a variety of essential biological processes, such as seed germination, shade avoidance and photomorphogenesis. In the bound form protein-chromophor interaction restricts the potentially possible degrees of freedom and guides the excited electronic state dynamics along a complex reaction coordinate involving both chromophore as well as protein contributions, including H-bond networks and protein bound water molecules (2). For a better understanding of these mechanisms for molecular reaction control we study the primary photochemistry of the bilin phycocyanobilin (PCB) in solution, employing fs transient absorption (TA) in the UV/Vis and mid-IR spectral region. The results of the TA measurements are complemented by quantum chemical calculations. In particular, PCB in solution shows conformational changes and indicates alteration of protonation state via photoexcitation, which is consistent with previous studies.(3,4,5)

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P88. Delivery of membrane proteins into small and giant unilamellar vesicles by charge-mediated fusion

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One of the current challenges in synthetic biology is the production of stable membrane mimetic systems and the insertion of components in these systems. We employ fusion of oppositely charged liposomes to deliver separately reconstituted membrane proteins into a common lipid bilayer, containing different proteins. After a systematic evaluation of different lipid compositions by lipid mixing and size distribution analysis, suitable conditions were further investigated for proteoliposome fusion. With this technique, we functionally co-reconstituted different terminal oxidases and ATP synthase from *Escherichia coli* into unilamellar vesicles. Successful fusion was confirmed by measuring ATP synthesis upon redox driven proton pumping.

Furthermore, reconstitution of proteins into giant unilamellar vesicles (GUVs) is still a challenging task and is an active field of research. We have inserted different protein populations into small positively charged liposomes and fused these with negatively charged GUVs. The insertion of different proteins into the same GUV was confirmed by fluorescence microscopy and functional analysis. The presented method is a mild and detergent-free alternative for membrane protein reconstitution into GUVs, even for complex multisubunit proteins into GUVs, maintaining protein orientation from the small liposomes. This method is a simple and versatile tool for membrane protein reconstitution to produce biomimetic systems with increased complexity (1).

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P89. Discrimination of the Redox active States in the Reductive Phase of Cytochrome c Oxidase

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Cytochrome c oxidase (CcO) is a membrane protein that catalytically reduces oxygen to water while pumping protons across the membrane. The sequential steps that go along with this redox driven proton translocation are still very much unknown due to the lack of experimental data. This is particularly true for the reductive phase of CcO that prepares for oxygen binding. We developed a technique to simultaneously bind carbonmonoxide (CO) as a ligand to heme a₃ and induce charge transfer by varying the electric potential under anaerobic conditions. FTIR spectroscopy reveals that the dissociation of CO by oxidation of heme a₃ is reversible upon reduction. Furthermore, it allows to disentangle the redox potentials of heme a and heme a₃ and even separates the redox potentials of first and second electron in the binuclear center, i.e. E and R states. The bound ligand at heme a₃ additionally monitors the oxidation state of heme a via the vibrational Stark effect. This technique allows us to discriminate the intermediates of the reductive phase, an already claimed but still controversially debated experimental achievement.

P90. Electron Transfer in DNA: Simulation Reaches a Microsecond Scale

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Long-range electron transfer (ET) in DNA has been in the focus of research interest for 25 years. In spite of that, the question of the underlying mechanism of DNA ET still awaits an unambiguous answer (1). Ab initio non-adiabatic simulation techniques allow for an unbiased description of ET (2), but such simulations are limited to tiny spatial and temporal scales. Our own previous efforts aimed to derive an efficient non-adiabatic QM/MM simulation scheme for ET, making nanosecond simulations of ET in realistic systems to routine (3).

Based on this previous work, we have developed a theoretical model in a bottom-up way. All of the

quantum-chemical as well as force-field calculations are substituted by theoretical models of the involved phenomena on a molecular level. A non-adiabatic simulation scheme is still employed, and no assumptions regarding the mechanism of ET are needed. Thus, the predictive power of the simulation is preserved, while pushing the limits of the accessible time scales beyond microseconds.

The model-based simulation scheme is applied to ET in various DNA species. Good agreement with the "full" QM/MM scheme is observed for the archetypal DNA hole transfer systems – the polyA sequence, and the sequences GTnGGG containing adenines as bridge sites. Further, hole transfer in larger, more complex DNA sequences is simulated, and the results are discussed.

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P91. Unusually Long-Lived Tyrosyl Radical in the Response to Red Light of an Animal-Like Cryptochrome

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Cryptochromes constitute a group of flavin-binding blue light receptors in various organisms. Recently, the response of cryptochromes to light was extended to nearly the entire visible spectral region on the basis of the activity of the animal-like cryptochrome aCRY in a green alga (1). This finding was explained by the absorption of red light by the flavin neutral radical as the dark resting state of the receptor, forming the anionic fully reduced state.

Time-resolved UV-vis spectroscopy using a flash photolysis setup with intensified CCD camera was performed on the full-length aCRY (2). The set-up was improved to overcome the challenge of measuring a slowly recovering system with nanosecond time resolution. A 750-ms pulsed stirring system as well as a horizontal probe light geometry was used, reducing the required amount of sample by a factor of ten compared to a previous flow system. The presence of a tyrosyl radical in aCRY was revealed 1 μ s after exciting the flavin radical with a red laser pulse. This tyrosyl radical exhibits an unusually long lifetime of 2.6 s and a red-shifted absorption maximum. Mutational studies disclosed the tyrosine 373 close to the surface to form the long-lived radical and to be essential for photoreduction. This residue and the lack of any response in secondary structure in the C-terminal extension as evidenced by FTIR spectroscopy differentiates aCRY from plant and fly cryptochromes. These findings imply that aCRY might have evolved a different signaling mechanism via a light-triggered sequence of electron transfers culminating in photooxidation of a yet unknown substrate or binding partner on the protein surface .

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P92. Sub-Microsecond Time-Resolved IR Spectroscopy: Proton Transfer and β -Sheet Response in Cryptochrome

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Plant cryptochromes are blue light receptors that regulate various central aspects of plant growth and development. Absorption of blue/UVA light leads to formation of the flavin neutral radical from the oxidized flavin via a decoupled electron and proton transfer (1). Little is known about the signal progression from the flavin to the C-terminal extension, which is essential for signaling.

Time-resolved step-scan and rapid-scan FTIR experiments were conducted at a spectral resolution of 4.5 cm⁻¹ and an excitation rate of 0.25 Hz. A time resolution of 500 ns was finally achieved by synchronizing single laser pulses from the 10 Hz repetition rate of the Nd:YAG / OPO laser system with the data acquisition. For the rapid-scan experiments, an electronic synchronization of the laser and interferometer mirror was implemented resulting in a 3D dataset covering a continuous range of more than 6 orders of magnitude in time up to 3.9 s. The first spectrum at 500 ns shows major contributions from the flavin anion radical, which is demonstrated to then be protonated by aspartic acid 396 to the neutral radical within 3.5 μs. The analysis revealed the existence of three intermediates characterized by changes in secondary structure. A marked loss of β-sheet structure is observed in the second intermediate evolving with a time constant of 500 μs. The only β-sheet is located within the α/β subdomain, ~25 Å away from the flavin pointing to signal progression by a protein quake (2). We have further investigated whether the proton transfer or the preceding ultrafast electron transfer are linked to the structural reorganization. For a mutant in which proton transfer is inhibited, time-resolved IR experiments point to a decoupling of proton transfer and β-sheet response.

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P93. Chromophore-protein interactions in Cph1Δ2 revisited: in vivo vs. in vitro holoprotein assembly

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Phytochromes utilize light to control various processes in plants, bacteria and fungi. These photoreceptors act as photochemical switches that interconvert between a normally inactive red (Pr) and an active far red (Pfr) absorbing state of the photoreceptor. Typical phytochromes have an N-terminal light sensing and a C-terminal output (transmitter) module, which also mediates protein dimerization [1]. The cyanobacterial phytochrome Cph1 [2] containing a phycocyanobilin chromophore has become a bona fide model system for phytochrome research, because it can easily overexpressed in *E. coli* and has an N-terminal chromophore-binding region, the sensor module, similar to plant phytochromes.

In a recent study we investigated the sensory module of Cph1 (truncated version Cph1Δ2) and labeled the protein with different pH-sensitive fluorescent dyes [3]. Light-induced protonation changes during the Pr → Pfr reaction were investigated by fluorescence lifetime changes of the surface bound pH-indicator dye SNARF [3]. The in vivo assembled Cph1 showed slightly different pH-dependent chromophore absorption bands [3] than the in vitro assembled protein [4]. We determined the pKa of chromophore deprotonation and analyzed the corresponding conformational changes in the protein by time-resolved fluorescence anisotropy measurements. Our data show that the chromophore-protein interactions in the in vivo assembled Cph1Δ2 shift the pKa of chromophore deprotonation by about 1 pH unit, indicating protein conformational plasticity.

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P94. Proton-coupled Electron Transfer at the Iron-Sulfur Cofactor of [FeFe]-Hydrogenases

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Iron-sulfur clusters are ubiquitous in biological electron transfer and redox chemistry. Most of the involved redox reactions are thermodynamically coupled to proton transfer events. In this study we

address the H₂ release reaction of [FeFe]-hydrogenases as a model system for proton-coupled electron transfer. Hydrogenases are iron-sulfur enzymes that catalyze hydrogen turnover in archaea, bacteria, and plants. [FeFe]-hydrogenases differ from other type of hydrogenases for their largely unbiased catalysis of both uptake and release of H₂. At a midpoint redox potential of –420 mV vs SHE hydrogen turnover is performed at negligible overpotential.

We report on pH and redox titrations of [FeFe]-hydrogenases from algae and bacteria as followed by real-time Fourier-transform infrared (FTIR) spectroscopy in attenuated total reflectance (ATR) configuration. Irrespective of origin and sample preparation the marker bands of the cofactor were found to be shifted to higher frequencies, indicative of a uniform decrease in electron density. Isotope exchange in combination with QM/MM calculation suggests this 'blue shift' to result from a protonated H-cluster, for example at the [4Fe4S] subsite. Electron injection proves the existence of a one-electron reduced state, H_{red}'⁺, which is characterized by an extra electron at the [4Fe4S] cluster. The 'blue shift' is observed for H_{red}'⁺ as well. Interestingly the H_{ox}/ H_{red}'⁺ midpoint redox potential for the blue-shifted species differs by more than 140 mV. These findings immediately support the presence of a 'regulatory proton' at the H-cluster. Introducing [FeFe]-hydrogenases as iron-sulfur model systems for proton-coupled electron transfer we present distinct proof for the influence of protonation on enzymatic redox chemistry.

Live Imaging and Optical Microscopy

P95. Resolving Interactions Inside Living Cells With Engineered Upconversion Nanoparticles

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Upconversion nanoparticles (UCNP) are efficiently excited by sequential multiphoton absorption of NIR light and emit photons in the UV/VIS regime and therefore can be detected with negligible background (1). Moreover, lanthanide resonance energy transfer (LRET) from UCNP to molecules in immediate proximity opens exciting possibilities as spectroscopic reporters or photoactuators with very high spatial resolution (2). However, even though strategies for improving the optical properties of UCNP emerged (3), the determinants of UCNP-based LRET as well as its application in a biological context are still poorly resolved. We have engineered biofunctional UCNP optimized for LRET as novel reporters for spatially-resolved protein interaction analysis within living cells. To this end, we implemented microscopic techniques for UCNP excitation and synthesized various nanoparticle species to systematically improve UCNP emission and energy transfer efficiency. These phenomena strongly profited from power densities far beyond commonly published values – in agreement with recent studies on this topic [3]. Strikingly, we observed further enhancement of LRET when breaking with traditional paradigms of UCNP design. In order to exploit these unique optical properties in a biological context, we established a biofunctional coating with an anti-GFP nanobody for selective targeting in the cytoplasm of living cells. With the mitochondrial TOM complex as model system we demonstrated specificity of UCNP functionalization by colocalization and further confirmed this interaction by LRET-sensitized dye emission.

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P96. In situ single cell pull-down for probing stability and stoichiometry of cytosolic protein complexes

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Investigation of composition, stoichiometry and interaction dynamics of signaling complexes is crucial for understanding the various signaling pathways. We have developed in situ single cell pull-down (SiCPull) for specific and efficient pull-down of protein complexes from individual cells, based on engineered, micropatterned functionalized surface architectures (1). Cells cultured on these surfaces are lysed by mild detergents, leading to almost instantaneous in situ capturing of GFP-tagged protein complexes, thus enabling the determination of their life-time, by monitoring the dissociation of prey proteins interacting with GFP-tagged bait proteins. Using SiCPull, we quantitatively determined the stability of various signal transducers and activators of transcription (STAT) complexes, ranging from seconds to nearly an hour. Strikingly, complex stoichiometry could be determined on the single cell level by single molecule imaging techniques. In combination with single cell manipulation, e.g. by microfluidics, this generic methodology provides robust possibilities to monitor stability and stoichiometry of cytosolic signaling complexes at different cellular states.

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P97. Development of an image correlation analysis to study the intracellular dynamics of nanoparticles

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Recent advances in nanoparticle (NP)-based drug delivery systems have pointed out the interactions at cellular and sub-cellular levels as key-factors for their efficiency. These interactions depend on the chemical-physical properties of the adopted NPs and affect their intracellular motion, which is commonly explored by fluorescence microscopy and spectroscopy techniques. However, none of these techniques has been specifically developed to characterize two-dimensional dynamics driven by flow terms, that are uniformly distributed within an angular range. Although these conditions are not deeply investigated in the current literature, they describe the effects of the interactions among biomaterials and cytoskeleton. To fulfill this gap, we present an Image Correlation Spectroscopy (ICS) analysis, arising from general concepts of SpatioTemporal Image Correlation Spectroscopy (STICS)(1) and extending the image Mean Square Displacement (iMSD) method(2). By overcoming some of the approximations adopted so far, the proposed approach simultaneously provides information about the strength of the driving speed and the net displacement of the ensemble, through a decoupling of the flow terms arising from the speed spatial distribution. Furthermore, the underlying symmetry of the investigated dynamics can be revealed, thus recovering geometric features which are commonly obtained by single particle analyses. Results from simulations show high stability in the measurement procedure and low sensitivity to background, leading to good characterization of motion under realistic experimental conditions. Finally, we give some examples of application exploring the intracellular trafficking of lipid-based NPs, in terms of diffusion coefficients, flow speed contributions over different regions of interest and velocity maps.

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P98. Single molecule imaging reveals dysferlin-mediated phosphatidylserine recruitment in membran repair

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Dysferlin (Dysf) has been proposed as a key protein for membrane repair in muscle cells, although the precise mechanism is still unknown. Dysf is a transmembrane protein, and rapid recruitment of Dysf to membrane lesions has been observed; direct observation of Dysf transport has so far remained elusive (1). Moreover, interaction of Dysf with various other proteins has been investigated for many years to understand its role in cell membrane repair, but studies addressing the role of phospholipids, the main components of the plasma membrane, are still scarce.

In this work, we have observed fast transport of plasma membrane-derived Dysf in living zebrafish by using fluorescence loss in photobleaching (FLIP) and single molecule imaging. In addition, in vivo imaging of human cells and zebrafish revealed that phosphatidylserine (PS) accumulates rapidly in the repair patch in a process mediated by Dysf, and single molecule trajectory analysis provided evidence that Dysf facilitates PS translocation from adjacent membrane regions to the site of lesion (2).

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P99. Investigation of ligand-receptor interactions at cell membranes via advanced fluorescence microscopy

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Cell communication and activation of signaling pathways highly depends on ligand stimulation of membrane receptor proteins. Fluorescent labeling is required to selectively observe these proteins of interest. Cells can be transfected with genes encoding the fusion of a fluorescent protein and the receptor protein of interest, which may lead to overexpression artifacts. Recent advances in targeted genome editing (CRISPR/Cas9) have greatly simplified endogenous labeling, which results in natural receptor densities in the expressing cells.

In this work, LRP6-mCherry or LRP6-mEosFP expressing HEK and H1703 cells were used to investigate the natural receptor density at the cell membrane and the interactions of LRP6 with its ligands DKK1 and Wnt3a. Colocalization experiments were performed with GFP-labeled DKK1. Dual-color images were acquired using a spinning disk confocal microscope, which technique allows high frame rates and minimal illumination of the sample.

Super-resolution photoactivated localization microscopy (PALM) offers single-molecule sensitivity so that individual receptors can be identified. Tracking single fluorescent particles with high time resolution enables the investigation of dynamic biological processes, such as the internalization of signalosomes, ligand binding to its receptor and recycling of components via vesicles. The single-molecule diffusional mobility shift assay (smDIMSA) (1) is based on PALM and exploits the reduced mobility of a membrane receptor upon binding a ligand. This method only requires labeling of the receptor protein but not the ligand. However, smDIMSA experiments require high receptor numbers for a good statistic of a single cell measurement.

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P100. Improving STED Microscopy by Focus Modulation

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STED microscopy has revolutionized the field of fluorescence microscopy by enabling in vivo imaging at resolutions below the diffraction limit. By using stimulated emission fluorophores can be locally switched off and, therefore, the fluorescence can be confined to a small subregion of the excitation volume. Despite the huge benefits STED microscopy has brought to the field of fluorescence imaging, switching off fluorophores by stimulated emission is never perfect. Accordingly, some fluorophores will escape depletion, and the confinement of the observation volume to a small subregion will be incomplete. This residual fluorescence will act as background superimposed on the super resolution image and consequently deteriorate the image quality. In this work we present a focus modulation scheme that allows to essentially remove all this unwanted background from super resolved STED images. Besides improving the image quality this advanced STED technique can also be used to facilitate the combination of 3D STED and fluorescence correlation spectroscopy where background by imperfect depletion is a fundamental issue.

P101. Superresolution microscopy of intrinsically fast moving flagellates

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Recent developments in superresolution microscopy made it possible to resolve structures in biological cells at a spatial resolution of a few nm and observe dynamical processes with a temporal resolution of ms to μ s. However, the optimal structural resolution requires repeated illumination cycles and is thus limited to fixed cells. For live cell applications substantial improvement over classical Abbe-

limited imaging can be obtained in adherent or slow moving cells. Nonetheless, to our knowledge a large group of cells, intrinsically fast moving flagellates, could not yet be addressed with superresolution microscopy. These include pathogens like trypanosomes, the causative agents of sleeping sickness in humans and Nagana in cattle. Attempts to immobilize these cells include drug treatment or embedding in agarose or gelatin gels. However, these methods either have unwanted side effects or are not sufficient for superresolution imaging because they do not efficiently suppress the flagellar beat.

Here, we present a novel hydrogel embedding and quantify its biocompatibility and immobilization efficiency. We characterize both the cells and the gel with respect to their autofluorescence properties and find them suitable for single-molecule fluorescence microscopy (SMFM). We apply SMFM to track individual Atto647N-labeled membrane proteins on the surface of immobilized trypanosomes and achieve a localization precision of 30 nm and a temporal resolution of 25 ms.

P102. Mechanism of left-right symmetry breaking during embryonic development in *C. elegans*

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The body plan of organisms typically is left-right (L-R) symmetric on the exterior but highly asymmetric in arrangement of the internal organs. This asymmetry is established during early stages of embryonic development and is crucial for the proper cell fate determination and organ development. The anterior-posterior (A-P) and the dorsal-ventral (D-V) symmetries need to be broken prior to establishing the L-R body axis. For example, the entry of a sperm in a *C. elegans* oocyte breaks the A-P symmetry and the point of entry becomes the posterior part before the first cell division in the embryo. Then, the symmetric division of posterior cell (P1) results in breaking the D-V symmetry as location of the daughter cells- AB and EMS, determine the dorsal and ventral side, respectively. The event that breaks the L-R symmetry at the 4 to 6 cell transition in *C. elegans* embryos has been previously recognized to be a directionally consistent and simultaneous skew or rotation of the daughter cells of AB. Recently, it was shown by Naganathan et. al (2014) that the skew is executed by chiral actomyosin flows. However, it is still unknown what sets the definite direction of the skew and how. In our current work, we investigate the relationship between chirality of the cortical flows and skewing of the cells. We report on the observation of an asymmetric distribution of cortical components on the dorsal and ventral surfaces of the embryo, which may be sufficient to generate the asymmetry required for a non-zero torque that skews the cells. This supports the predictions of our theoretical model and sets the direction for a full mechanistic description of the event. We also explore the role of cell-cell interactions at the cell junctions in determining the extent of the skew.

Synthetic Biology and Nanobiophysics

P103. Electrobiophysical study of Self-assembled SWNT/DNA hybrid & electroactive nanostructure

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DNA self-assembled hybrid nanostructures are widely used in recent research in nanobiotechnology. Combination of DNA with carbon based nanoparticles such as single wall carbon nanotube (SWNT), multi wall carbon nanotube (MWNT) and carbon quantum dot applied in important biological applications. Many examples of biosensors, nanowires and nanoelectronic devices, nanomachin and drug delivery systems fabricated by these structures. In this study, a new hybrid nanostructure has been fabricated by non-covalent interactions between single and double stranded DNA and SWNT nanoparticles and studied biophysical properties of these structures comparatively. Biophysical properties of hybrid nanostructure studied by circular dichroism, UV-Vis and fluorescence spectroscopy techniques. Also, electrochemical properties of hybrid nanostructure studied by cyclic voltammetry, linear sweep voltammetry, square wave voltammetry chronoamperometry and impedance spectroscopy (EIS). Results of this investigation revealed that the biophysical and electrochemical properties of SWNT/DNA hybrid nanostructure were different in compare to ss-DNA, ds-DNA and SWNT singly. Circular dichroism study of DNA/SWNT showed the first experimental confirmation that ss-DNA enters on sidewall and central hole on SWNT. Some nucleic acids bind to and wrapped around the nanotubes through π - π stacking interactions. The results indicated a reduction in the peak intensity of CD and UV-Vis spectrum after adding SWNT to ss-DNA and ds-DNA. Electrochemical experiments indicated that the modification of single-walled carbon nanotubes by ssDNA improves the electron transfer rate of hybrid nanostructure. This was demonstrated DNA/SWNT hybrid nanostructure should be a good electroactive nanostructure that can be used for electrochemical detection or sensing.

P104. Controlling structural changes in extruded fibronectin nanofibers

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Fibronectin nanofibers are a major component in the extracellular matrix (ECM). They play an important role in cell adhesion and cellular mechanosensing. Binding to cell surface receptors and the application of cellular forces, which partly unfold the fibronectin molecules, induces fibrillogenesis in vivo.

We have introduced a new approach involving water-based extrusion of protein solution to study fibronectin fibrillogenesis in vitro. (1) A fibronectin solution was extruded through nanoporous aluminum oxide membranes (AOM) in physiological buffer. The morphology of extruded nanofibers was analyzed using scanning electron microscopy. Our simple, one-step extrusion process yielded bundles of fibronectin nanofibers up to millimeter in length with a unique diameter range from only 15 nm for single nanofibers to several micrometers for fiber bundles. Fiber dimensions were adjusted by tailoring the fibronectin concentration and AOM pore diameter, which yielded diameters of extruded fibers, which were in the range of native fibronectin fibers.

Further on, we used confocal fluorescence microscopy and Förster resonance energy transfer measurements to study conformational changes in extruded fibronectin fiber bundles. We found that extrusion through nanoporous membranes induces lasting structural changes in fibronectin fibers in aqueous solution. Most importantly, by tailoring the fibronectin concentration and AOM pore diameter we were able to reproducibly control the degree of fibronectin unfolding in extruded fiber bundles.

In summary, we established a new model system for fibronectin fibrillogenesis in a non-cellular environment, which closely mimics native fibronectin unfolding. This well-controllable fiber preparation method will open up new avenues in synthetic biology and enable us to study the underlying principles

of cellular mechanosensing under physiological conditions.

(1) Raoufi, M. et al., Nano Lett 15, 6357–6364 (2015).

P105. Mechanics of Giant Lipid Vesicles by Indentation with a conical AFM-Tip

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Cell mechanics have an enormous impact on many biological processes. For instance it influences exo- and endocytosis and contributes to cell adhesion and migration. The mechanical behaviour of cells itself is mainly controlled and determined by the contractile cortical cytoskeleton and its connection to the plasma membrane which is fulfilled by a variety of proteins. To shine more light on these mechanical behaviours we investigated a cell mimetic model system made up of giant unilamellar lipid vesicles. The mechanics of this system was studied by indentation with a conical atomic force microscopy tip. For this purpose the giant vesicles were immobilized to a solid support by the avidin-biotin interaction. The indentation was performed in the centre of the vesicle, optically controlled by an inverted fluorescence microscope. The pre-stress of the bilayer T_0 and the area compressibility modulus K_A were extracted from the nonlinear force indentation curves by computing the contour of the vesicle at a certain force. Values of the area compressibility modulus we obtained are in the order of 0.04 N m⁻¹ corresponding well to results obtained by micropipette suction and by investigation of membrane undulations by other groups. Polymerization of an artificial actin shell inside the liposomes significantly stiffens the vesicles, accompanied by an increase in the area compressibility modulus. Thus, this nonlinear membrane theory neglecting the influence of membrane bending describes the mechanical response of the vesicles with sufficient accuracy.(1)

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P106. Fluorogenic Riboswitch Engineering with Microfluidics

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In this conference contribution we present an approach for engineering fluorogenic glycine riboswitches. For the design of functional riboswitches, a three-part RNA approach was applied based on the idea of linking a RNA sensor, transmitter, and actuator part together. For the RNA sensor and actuator part, we used the tandem glycine aptamer structure from *Bacillus subtilis*, and fluorogenic aptamer Spinach, respectively. To achieve optimal signal transduction from the sensor to the actuator, a riboswitch library with variable transmitter was screened with a microfluidic large-scale integration chip. This allowed us to establish the complete thermodynamic binding profiles of a riboswitch library with a size of 120 constructs with only one chip experiment. Glycine dissociation constants of the top 12 strongest fluorescence response riboswitches varied between 99.7 - 570 μ M. Furthermore, the kinetic glycine binding (k_{on} , and dissociation (k_{off}) rates, and corresponding energy barriers of the 12 strong fluorescence response riboswitches were determined with the same chip platform. Here, the k_{on} and k_{off} were in the order of 10⁻³ s⁻¹ and 10⁻² s⁻¹, respectively. Conclusively, we demonstrate that systematic screening of synthetic and natural linked RNA parts with microfluidic chip technology is an effective approach to rapidly generate fluorogenic metabolite riboswitches with a broad range of biophysical response properties. In a last step we demonstrate that in vitro engineering fluorogenic riboswitches are applicable for real time imaging of glycine fluxes in bacteria.

Protein-Lipid Interactions

P107. Interactions of collybistin II with phosphoinositide species (PIPs) on solid supported lipid bilayer

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The formation of active zones inside the postsynaptic membrane is a key step in synaptogenesis. Inside these active zones, neurotransmitter receptors (glycinergic & GABAergic) accumulate. This process results from interactions of the proteins neuroligin-2, gephyrin and collybistin.(1)

Collybistin is a brain specific guanine nucleotide exchange factor (GEF) and consists of a Dbl homology domain (DH) connecting the C-terminal pleckstrin homology (PH) domain with a SRC 3 homology (SH3) domain at its N-terminus.(2) The terminal domains associate in an intramolecular fashion leaving the protein in a closed conformation. In this auto-associated state collybistin is unable to interact with receptor lipids inside the postsynaptic membrane. This was confirmed by Ludolphs et al. who measured the full-length collybistin and an isoform lacking the SH3 domain on phosphatidylinositol phosphate (PIP) containing solid supported lipid bilayers (SLBs) by reflectometric interference spectroscopy (RIfS).(3)

Further investigation of collybistin's activation mechanism is necessary to generate a better understanding of the processes in and underneath the postsynaptic membrane. Thus, we isolate different isoforms of collybistin and determine their interactions with PIP in vitro. The measurements are performed on PIP doped SLBs in either a mechanical way by atomic force microscopy (AFM) or a non-invasive, optical way via RIfS. Both techniques will show changes in protein topology upon the SLB's surface and allow conclusions to conformational changes induced by the different PIP species and collybistin isoforms used.

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P108. Membrane Lateral Pressure Regulates Dipolar Relaxation Dynamics at the Active Site of an ATPase

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Copper is an essential cofactor for redox enzymes but toxic when it accumulates in the cell. Copper homeostasis relies on transmembrane Cu⁺ transporting PIB-type ATPases. Molecular Dynamics simulations of the Cu-ATPase CopA from *Legionella pneumophila* (1) (LpCopA) suggest a water-accessible Cu⁺-binding site at the conserved CPC motif in the E2.Pi intermediate, where Cu⁺ occlusion is expected from homology with the Ca²⁺-ATPase, SERCAII (2,3). However, the role of the additional physical constraints imposed by the biological membranes on LpCopA is not known. We have labeled the transmembrane cysteines of the copper-binding site with the polarity-sensitive fluorophore 6-bromoacetyl-2-dimethylaminonaphthalene (BADAN). Time-resolved dipolar relaxation studies of the dye show that membrane lateral pressure affects the hydration and dipole mobility of the ion binding site with high topological discrimination. The data demonstrate that the lipidic phase can contribute to the energetics of the ion transport cycle by providing lateral forces that affect transient hydration and dehydration events at the ion-binding site.

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(3) J. Møller et al. 2010, *Biophys.Q. Rev.* 43, 501–566

P109. Azido-modified Lipids: Miscibility with Phospholipids and the Use to Study Peptide-Lipid-Interactions

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For studying interactions between membrane peptides and lipids various methods are available. In the last years, chemical cross-linking combined with MS has matured into an alternative method to study these interactions. Using the cross-linking/MS approach, information about the three-dimensional structure of peptides/proteins can be obtained. For photo-cross-linking, different cross-linkers are currently employed, such as benzophenones, phenylazides, and diazirines. These are activated by UV-irradiation leading to the formation of very reactive species, which will react non-specifically with amino acid residues in peptides/proteins. The great advantage of the cross-linking/MS approach is that membrane proteins can be investigated in a native-like environment.

Beside the modification of isolated peptides/proteins with different cross-linkers, membrane lipids can be modified. The idea to incorporate photochemical cross-linkers into lipids is not entirely new. However, previous work regarding phospholipids that contain photoactivatable precursors relies on a comparable physicochemical behavior of modified and classical membrane lipids. Alternatively, it is assumed that both lipids exhibit a virtually perfect miscibility.

We have shown previously that the introduction of an azido group in membrane lipids might lead to the formation of lamellar aggregates where the lipids are arranged in an interdigitated fashion. In this study, we systematically evaluate the miscibility of azidolipids with classical phospholipids using TEM, DSC, and FTIR spectroscopy. Also, the long-term stability of mostly unilamellar vesicles prepared from azidolipid-phospholipid-mixtures was examined by DLS. One mixture was further used for photochemically induced cross-linking experiments to demonstrate the capability of our azidolipids for conducting interaction studies with membrane peptides/proteins. The cross-linking process was followed using ESI- and MALDI-MS as well as ATR-IR spectroscopy.

P110. Annexin A2 in the formation of lipid microdomains.

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Lipid microdomains regulate cellular processes by serving as organizing centers, affecting the assembly of signaling molecules and membrane trafficking (1-2). Annexin A2 (AnxA2 WT) is a peripherally Ca²⁺ regulated membrane binding protein that can induce lipid domain formation and plays a role in exocytosis and endocytosis (3). In order to better study the mode of annexin-membrane interaction, we generate two mutants of the AnxA2 protein that showed amino acid substitutions in residues predicted to be involved in lateral protein-protein interaction of membrane-bound AnxA2. The proteins were purified and their lipid binding was first characterized by co-pelleting with liposomes consisting of POPC:POPS:Cholesterol at a ratio of 60:20:20. All mutants showed binding to the negatively charged liposomes in the presence of Ca²⁺, suggesting that the lateral modifications of the protein do not affect the ability to bind to the membrane. To characterize a potential effect of the mutations or AnxA2-induced lipid domain formation and AnxA2 clustering at the membrane, we carried out GUV binding and crosslinking experiments. Results will be discussed.

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P111. Force Field Comparison: Protein-lipid interactions at the membrane interface.

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Molecular Dynamics (MD) offers the possibility to study biological processes in molecular detail often not reachable by experiments. In consequence, a great number of force field parameters have been developed to describe a wide variety of molecules ranging from inorganic ligands and small organic

molecules over proteins and lipids to nucleic acids.

Typically, these parameters have been established and extensively evaluated to reproduce molecular characteristics of individual compounds, i.e. soluble proteins or lipid bilayers. However, mostly due to missing experimental data to compare to, the properties of compound mixtures (i.e. protein-membrane systems) have been less well tested. Nevertheless, those are of particular interest, given that biological systems generally involve an extensive mixture of molecules. The selection of a force field or their combination to simulate such a system is a difficult task, since different force fields were built to reproduce a variety of system properties. Moreover, the subtle balance of the force field parameters may get strongly influenced by the presence of other compounds in the system.

Here, the ability of four force fields suitable to simulate protein-membrane systems, namely the united-atom GROMOS 54a7, the all-atom CHARMM36, and the two all-atom combinations Amber14sb/Slipids and Amber14sb/Lipid14, to reproduce the experimental insertion energies of Whimley-White peptides into the membrane interface is compared. Although the overall performance of each of these force fields is satisfactory, significant differences in the prediction of the insertion energy of individual amino acids were observed. In consequence, different biophysical behavior of transmembrane and peripheral membrane proteins is expected.

P112. Annexin-Phospholipid interactions in artificial membrane systems

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Biological membranes are a patchwork of lipids and associated proteins with essential functions not only in providing a physical barrier and compartmentalization but also in membrane trafficking events and signaling. The dynamic formation of membrane microdomains in spatially and temporally depends on the interplay between lipids and proteins and enables cells to controlled shape and intracellular processes. Of particular importance, associated proteins can specifically interact with certain membrane lipids and affect their local distribution. The annexins are a family of such membrane binding proteins that can induce the formation of cholesterol and phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) rich membrane microdomains in artificial and cellular membranes. Here we performed a quantitative analysis of the annexin A1 / A2 (AnxA1/AnxA2)-membrane interaction using the Quartz Crystal Microbalance with Dissipation (QCM-D) on a solid supported bilayers (SLBs) containing biologically important lipids, such as (PI(4,5)P₂) and cholesterol. We also designed a new crosslinking reagent for mapping possible intermolecular interactions in membrane bound annexins.

P113. Polarized ATR-FTIR Spectroscopy to Study α -Synuclein Membrane Interaction

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The aggregation of α -synuclein causes different forms of Parkinson's disease. In solution α -synuclein is intrinsically unordered but undergoes structural changes upon interaction with cell membranes. This process plays a crucial role in the aggregation of α -synuclein and the formation of fibrils. Attenuated total reflection (ATR)-FTIR spectroscopy is a very sensitive tool to investigate the dynamics of protein interaction. A solid supported bilayer (SSLB) is placed on the silicon crystal to simulate a cell membrane. With this technique we are able to investigate changes in the lipid bilayer and in the protein simultaneously. Using polarized infrared light we are able to determine the orientation of certain infrared active groups with respect to the surface normal and make conclusions about the orientation of different structures. This gives new insights into the aggregation process and potential α -synuclein induced pore formation in membranes.

P114. Lipid-Protein Interactions and the Influence on Membrane Dynamics

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Biological cell membranes are crucial for living organisms. A major part of these cell membranes is constituted of proteins and lipids. Their interactions effect a variety of important cell function as i.a. the regulation of molecular and signal transport which are strongly coupled to structure, composition and dynamics of the membrane.

Neutron scattering is a powerful tool to probe structure and dynamics of biological membrane mimics on an atomistic scale [1,2]. This allows studies on e.g. the diffusion of lipids, the undulation motions of membranes or also the mutual interactions of the membrane constituents. Using the backscattering spectrometer IN16B (ILL, Grenoble, France) quasielastic neutron scattering (QENS) experiments were performed on phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) vesicles at 310 K with and without the transmembrane sequence of the transferrin receptor protein. Thereby, protonated and deuterated DMPC molecules were used to study the influence of the protein on the lipid dynamics and the protein dynamics as well.

The measurements of the neutron scattering experiments indicate a restricted lipid mobility for the lipid-protein vesicles compared to the pure lipid vesicles. First results and the analysis of the fundamental question how far the influence of the proteins reaches on the surrounding lipids will be presented in this contribution.

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P115. Organisation of the lipopeptide Lipobiotin and interaction with human and bacterial model membranes

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Lipobiotin is a biotinylated analogue of the Toll-like receptor activating lipopeptide Pam3Cys-SK4, which is used to label mycobacteria for a final purification of pathogen-containing organelles from primary cells (1).

Proceeding from this application, we try to generate understanding of the underlying molecular processes to allow new and more specific applications in the field of binding, labelling and separating pathogens.

Our strategy is the complementary use of different state-of-the-art biophysical techniques for the characterisation of the biological system, in particular of model membranes.

The unique structure of Lipobiotin aggregates was investigated using SAXS and light scattering. Reconstituted Lipobiotin monolayers imaged with AFM showed the formation of aggregates and clover shaped domains depending on lateral pressure.

Fluorescence spectroscopy, film balance experiments and imaging of giant liposomes revealed a binding of Lipobiotin to the membranes along with aggregation of the liposomes, both with moderate lipid specificity. In contrast, an intercalation of Lipobiotin and the inducement of liposome fusion occurred with high lipid specificity.

Our results obtained from experiments with model membranes mimicking human cell membranes, membranes of apoptotic vesicles and bacterial membranes can explain the preferential labelling of bacteria and apoptotic vesicles by Lipobiotin which has been reported recently (2). Further, the possibilities and limits of the different biophysical techniques and the power of our multi-methodical approach are demonstrated.

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P116. Reconstitution and characterization of the chemokine receptor CXCR4 monitored by PWR and MST methods

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The chemokine receptor CXCR4, which is expressed predominantly on T cells and macrophages, is a highly relevant pharmacological target because of its involvement in inflammatory disorders, cardiovascular diseases, cancer and HIV infection. Recent crystal structure of CXCR4 (1), afforded a great advance in understanding of receptor activation mechanisms. Yet, direct observations of a receptor in a real time and in a biologically relevant context are required for a detailed understanding of the dynamic nature and intricate balance of ligand-receptor interactions. To elucidate this aspect, we purified the wild type CXCR4 from a mammalian cells using HALOtag system (Promega). The stability and activity of the purified CXCR4 was determined by micro scale thermophoresis (Monolith NT.115, NanoTemper Technologies) and nanoDSF (Prometheus NT.48, NanoTemper Technologies). Next, CXCR4 was incorporated into supported lipid bilayers of varying lipid compositions and the receptor-ligand interactions monitored by the plasmon waveguide resonance spectroscopy (PWR).

PWR method is very sensitive (femto mole quantities of material can be detected) and label-free, which is of great advantage for studies of the influence of lipid composition on the ligand binding, on the activity and the conformation state of the receptor. PWR has already been successfully applied by I. Alves to monitor the activation of several GPCRs (like rhodopsin, opioid, neurokinin receptors) (2,3). Our preliminary data unambiguously show that with the use of HALOtag based purification method one can easily access sufficient quantities of the functional wild type CXCR4 for subsequent label-free biophysical studies.

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P117. Interactions of Cationic Peptides with Lipid Bilayers - Influence of Peptide and Lipid Modifications

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The upcoming resistance of bacterial strains against antibiotics is one central issue of the 21st century. In contrast to the specific mechanism of action of antibiotics, antimicrobial peptides (AMPs) show an unspecific interaction with bacterial membranes. AMPs show a high abundance of charged and hydrophobic amino acids, leading to a cationic and amphipathic structure. Since the complexity of bacterial membranes is too high, model membranes with negatively charged phospholipids, e.g. PG, CL or PS are often used to study specific interactions with peptides.

We investigate interactions of small cationic peptides of defined charge and hydrophobicity with phospholipid model membranes with respect to structure formation and molecular orientation of both components. The peptides, composed of five lysines alternating with uncharged amino acids X of increasing hydrophobicity (from G up to L) were designed to tune the peptide hydrophobicity.

The hydrophobicity of (KX)₄K peptides determines the stability of the β -sheets bound to DPPG bilayer. For hydrophilic G unordered conformations are detected. For X = A or Abu, the secondary structure changes with temperature, whereas for X = L or V, stable β -sheets are formed at all temperatures. The bound β -sheets strongly increase the phase transition temperature T_m of DPPG (1).

All other tested negatively charged lipids show general upshift of T_m to higher temperature when mixed with (KL)₄K. The gel phase was more stabilized for lipids with smaller headgroups, e.g. TMCL, than for lipids with larger headgroups, e.g. DMPS. A direct correlation between the critical packing parameter and the extent of the upshift of T_m was found for all studied negatively charged lipids.

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P118. Membrane mediated modulation of DEG/ENaC ion channels by amphiphilic compounds

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The Degenerin (DEG)/Epithelial Na⁺ Channel (ENaC) family of ion channels comprises in mammals four genes encoding subunits of the Acid Sensing Ion Channels (ASIC), four genes encoding ENaC subunits and one gene encoding the Bile Acid Sensitive Ion Channel (BASIC).

BASIC is gated by amphiphilic compounds like bile acids or artificial detergents, however it is not known if other mammalian DEG/ENaC family members are affected by similar molecules. To address this question, we analyzed how amphiphilic compounds modulate ASIC1a, ASIC3 and $\alpha\beta\gamma$ ENaC in *Xenopus laevis* oocytes. ASIC currents were potentiated by the detergents sarkosyl, Triton X-100, octyl glucoside and the fenamate flufenamic acid. In contrast the antipsychotic drug chlorpromazine inhibited ASIC currents. Interestingly, all compounds inhibited $\alpha\beta\gamma$ ENaC currents.

If a membrane dependent mechanism was underlying these effects, we reasoned that the well-studied membrane dependent ion channel gramicidin A and other ion channels like the purinergic receptor P2X2 would be modulated by these substances, too. Indeed, most compounds altered both, Gramicidin A and P2X2 currents.

To gain further insight into the compound-membrane interaction DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) membrane stacks with compounds incorporated were analyzed by large angle x-ray diffraction experiments. In line with our electrophysiological results we observed that substances, which increased channel activity positioned in the lipid tailgroup region and substances, which decreased currents accumulated in the lipid headgroup region.

Finally, we show that micromolar concentrations of antidepressants inhibited ASICs and P2X2 in a similar fashion and most likely act via a membrane dependent mechanism as well. In summary, we demonstrate that DEG/ENaC channels can be regulated by a range of structurally diverse compounds, which all interact with membranes.

P119. P2X-receptor antagonists as inhibitors of α -toxin binding and toxicity

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The α -toxin from *S.aureus* is one of the most-studied toxins. Still, the basic mechanism of membrane-protein interactions is poorly understood. The toxin is able to lyse pure lipid membranes if they contain either sphingomyelin or phosphatidylcholine, since these two lipids seem to constitute the primary binding partner. Concentrations necessary to lyse liposomes are 200 nM and above, depending on the composition. In particular, sphingomyelin in the liquid-disordered phase increase oligomerisation efficiency (Schwiering 2013). In contrast, certain cells are lysed at low or sub-nanomolar concentrations. In the last years experimental evidence is accumulating which points towards a role of ATP-gated P2X-channels in enhancing lysis (Skals 2011). Is this concept a general one in the context of toxin-induced lysis? A study of the effect of various P2X-receptor antagonists on hemolysis induced by α -toxin from *S.aureus* showed that the inhibitory concentrations of antagonists like PPADS, MRS2159 and BBG are in a similar range as reported for other erythrocytes and other toxins. However, treatment with hexokinase, oxATP or ATP did not alter lysis nor did changes in extracellular calcium concentration. Furthermore, inhibitors PPADS and MRS2159 interfere also with the interaction of the toxin with pure-lipid membranes, as evidenced by efflux-measurements, gel-electrophoresis and fluorescence microscopy and PPADS inhibits binding of Hla to HaCaT cells. Thus, these results point more towards lipid/toxin/membrane effect than towards an involvement of P2X7-receptors in the action of α -toxin induced hemolysis.

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P120. Interaction of the fatty acid moiety of muraymycin antibiotics with lipid bilayers

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The naturally occurring Streptomyces-produced muraymycins are a class of nucleoside antibiotics. The activity of muraymycins has been shown to be correlated to the length and terminal group of a fatty acid moiety attached to the muraymycin backbone (1). As the target of these antibiotics is the intracellular membrane protein MraY, penetration of the plasma membrane is a prerequisite for effective cell wall synthesis inhibition. The structural motif of the lipid sidechain (LSC) is therefore believed to be involved in cellular uptake. To investigate the influence of the fatty acid moiety on the interaction of muraymycins with membranes, a simplified model system based on fluorescently labeled LSC and immobilized giant unilamellar vesicles was chosen. This allows the direct observation of the interaction of the labeled LSC with the lipid bilayer by confocal microscopy. A previous study showed an accumulation of the LSC of muraymycin-A1 in the lipid bilayer and a significant uptake into the vesicles, suggesting a reduction of membrane integrity by accumulated LSC (2). Our work further investigates this effect and focuses on the influence of the highly charged terminal group on the interaction with membranes by employing several LSC derivatives with decreasing basicity at the ω -position. The influence of membrane composition is further elucidated by variation of lipid headgroups and introducing a physiologically relevant negative charge in the lipid bilayer.

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P121. Anisotropic metal growth on phospholipid nanodiscs via lipid bilayer expansion

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Self-assembling biomolecules provide attractive templates for the preparation of metallic nanostructures. However, the intuitive transfer of the “outer shape” of the assembled macromolecules to the final metallic particle depends on the intermolecular forces among the biomolecules which compete with interactions between template molecules and the metal during metallization. The shape of the bio-template may thus be more dynamic than generally assumed. Here, we have studied the metallization of phospholipid nanodiscs which are discoidal particles of ~10 nm diameter containing a lipid bilayer ~5 nm thick. Using negatively charged lipids, electrostatic adsorption of amine-coated Au nanoparticles was achieved and followed by electroless gold deposition. Whereas Au nanoparticle adsorption preserves the shape of the bio-template, metallization proceeds via invasion of Au into the hydrophobic core of the nanodisc. Thereby, the lipidic phase induces a lateral growth that increases the diameter but not the original thickness of the template. Infrared spectroscopy reveals lipid expansion and suggests the existence of internal gaps in the metallized nanodiscs, which is confirmed by surface-enhanced Raman scattering from the encapsulated lipids. Interference of metallic growth with non-covalent interactions can thus become itself a shape-determining factor in the metallization of particularly soft and structurally anisotropic biomaterials.

P122. Lipid clustering by antimicrobial polymers and bacterial lectins

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The current urge to understand the role of lipids in defense against pathogens is driven by two strategies: killing pathogens and protecting the cell from infection.

Antimicrobial peptides and antimicrobial polymers offer a promising alternative to classical antibiotics through their action on membrane integrity. Thus, there is a wealth of studies on the influence of membrane active compounds, their charge, valence, hydrophobic/hydrophilic ratio, specific and unspecific binding properties. However, much less is known so far on the implications of lipid compositions.

Similarly, attention has been paid to lectin proteins, which are promising targets for drug development. They can initiate uptake of pathogens into host cells. Again, the existing studies focus on the properties of the protein, rather than the role of the recognized lipid species or the composition of the

matrix membrane.

Common to lectins and antimicrobial peptides and polymers is the ability to cluster lipids through lipid selection and recruitment from a mixed membrane.

We employ an array of methods: microcalorimetry such as DSC can detect clustering, ITC yields binding properties. Langmuir monolayers help to identify conditions for binding, and to distinguish electrostatic and hydrophobic contributions to the interaction. TCSPC fluorescence will serve advanced leakage studies, and characterize lipid mobility. Advanced IR-spectroscopy (ATR and IRRAS) will aid to detect interaction types, properties of lipids and the clustering agent, and simultaneously provide information on orientations.

P123. Influence of Model Peptides on the Main Phase Transition Temperature of Lipid Bilayers

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Lipid bilayers melt from the gel phase to the fluid phase upon heating at the so called main phase transition temperature. This melting point can be shifted upon the insertion of proteins (1). The hydrophobic matching theory predicts an upward shift of the melting temperature in the case that the protein's hydrophobic length exceeds the bilayer's hydrophobic thickness (positive mismatch) (2). Vice versa, a downward shift of the melting temperature shall be introduced by negative mismatch. In order to test this theory and to discover other determinants of the melting behaviour of biomembranes, coarse-grained molecular dynamics simulations of the melting of pure membranes and of membranes with embedded model peptides of different characteristics were conducted. Spontaneous gel phase formation of pure phospholipid bilayers results in different gel qualities with only slightly varying lipid order, which is preserved during further equilibration. Upon heating, the melting temperatures of these pure membranes are strongly dependent on the initial gel quality, showing differences of 20 K for one lipid type. The model peptides change the order of lipid bilayers in their vicinity. In the gel phase, all peptides cause a bilayer disordering. The extent of membrane disordering is thereby depending on the peptides' hydrophobic length and chemical nature. It furthermore correlates with the melting temperature of the host membrane for each particular system.

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Channels and Transporters

P124. Intermolecular interactions in the activation of Two Pore Channels

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Two-pore channels (TPCs) are intracellular cation channels that are widely expressed in eukaryotic cells. In human cells and in plant cells of *Arabidopsis thaliana*, hTPC2 and AtTPC1 are embedded in the membrane of their acidic compartments, the lysosomes and vacuoles, respectively. Depending on the host cell, they are involved in diverse processes like the cellular cation and pH homeostasis (1), Ebola virus infection (2) and long-distance cell-cell signalling (3). The gating mechanism and regulation of these homodimeric channels are therefore of strong interest. It was shown that AtTPC1 gets activated upon binding of Ca^{2+} to the EF-hands with the second EF-hand being essential for channel activation by depolarization (4). Furthermore, patch-clamp experiments with wild type and truncated variants demonstrated that the C-terminus of AtTPC1 is an indispensable player for channel activity (5). In contrast, the homologous TPC2 of humans is gated open upon addition of phosphoinositides (PI(3,5)P₂) (6,7), however, the exact binding site and the relation to channel activation are unknown. To investigate the mode of channel activation of AtTPC1 and hTPC2 we combined experimental techniques and molecular dynamics simulations at the coarse-grained and atomistic level. Results demonstrated that AtTPC1 subunits interact via their C-terminal regions, and PI(3,5)P₂ lipids tend to bind to predominately positively charged sub-regions of hTPC2. Further experiments will show if these homologues share common features in the gating mechanism.

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P125. K⁺ ions in the selectivity filter serve as voltage sensor in potassium channels

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The control of ion channel activity is mainly achieved by sensing domains, which perceive regulatory factors. They undergo a conformational change, which is then propagated to the pore domain. To understand this dynamic interplay between sensor domains and the gates in the pore domain, it is important to properly understand the gates in isolated K⁺ channels.

The viral Kcv channels are such pore-only K⁺ channels and ideally suitable for addressing this question. In spite of their simplicity one of their gating processes is voltage-dependent. It is modulated by both the external K⁺ concentration and the K⁺ gradient. The rate constants correlate with the voltage-dependent probability of three ions being in the filter instead of two. This strongly suggests that the selectivity filter can function as a voltage-sensitive gate, in which the permeating ions are the "voltage sensor".

The proposed filter gate is not independent from the rest of the protein; we can influence it with mutations near the cytosolic mouth of the channel. This means that the filter gate is accessible to the regulation by sensor domains, which typically connect to the pore near the inner gate region. Since the selectivity filter is so highly conserved in K⁺ channels, the results have a general implication on the understanding of channel regulation.

P126. Two amino acids form a novel cytosolic gate in viral potassium channels

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Kcv channels from Chlorella viruses are tetrameric, pore-only potassium (K⁺) channels (78-120 aa per monomer). They exhibit the typical architecture of K⁺ channel pores with two transmembrane domains, a pore helix and a selectivity filter. Here we exploited the genetic variability among these channels to uncover a novel mechanism of an inner gate. To this end, we compared two different channels, KcvNTS and KcvS, which share a sequence identity of 87%. KcvNTS has a mean open probability (Po) of 0.9 whereas KcvS shows a Po of 0.4. Dwell-time analyses reveal that this difference is mainly caused by a long lasting closed state, which is present in one and not in the other channel.

To understand the impact of the few amino acid differences on gating, we generated a set of mutants and examined their activity in planar lipid bilayers. The differences in gating can be fully attributed to a motive of two amino-acids (GF in KcvNTS vs. SF in KcvS) near the cytosolic entrance to the pore. The phenylalanine forms the actual gate. It can obstruct an ion passage through the cavity, which is reflected by the long lived closed time. The amino acid one position upstream controls the orientation of the Phe side chain and thus the gating. Further experiments and structural considerations support the hypothesis that the orientation of the Phe side chain is controlled by an H-bond between the serine side chain and the preceding helix turn. This weak chemical interaction alters the alpha-helical bending angle, which in turn orients the phenylalanine side in the ion pathway. The fact that Ser (and Thr) residues are generally enriched in alpha-helical transmembrane segments [1] suggest that such an intrahelical interaction can participate in molecular mechanisms, which are underlying protein dynamics like ion channel gating.

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P127. Discovery of a new voltage-gated proton channel

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The Hv1 voltage-gated proton channel plays an important role as part of the cellular proton extrusion machinery and is essential for charge compensation during the respiratory burst of phagocytes. It has been identified in a wide range of eukaryotes throughout the animal kingdom, with the exception of insects. Therefore, it has been proposed that insects do not possess an Hv1 channel. We report the existence of an Hv1-type proton channel in insects [1]. We searched insect Transcriptome Shotgun Assembly (TSA) sequence data-bases and found putative Hv1 orthologues in various polyneopteran insects. To verify that these putative Hv1 orthologues were functional channels, we studied the Hv1 channel of *Nicoletia phytophila* (NpHv1), an insect of the Zygentoma order, in more detail. NpHv1 comprises 239 amino acids and is 33 % identical to the human proton channel hHv1. Patch-clamp measurements in a heterologous expression system showed proton selectivity, and pH and voltage-dependent gating. Interestingly, NpHv1 shows slightly enhanced pH-dependent gating compared to the human channel. Mutations in the first transmembrane segment at position 66 (Asp66), the presumed selectivity filter, lead to a loss of proton-selective conduction, confirming the importance of this aspartate residue in voltage-gated proton channels [2].

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P128. pH-dependent gating mechanism of the viral potassium channel KcvNTS

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With only 82 amino acids per monomer, the viral potassium channel KcvNTS forms a functional homotetramer, resembling the pore region of more complex potassium channels. Despite its small size, KcvNTS possesses all essential properties of potassium channels and is therefore a useful tool for structural and functional analyses. pH-dependencies are already known for certain potassium channels, such as KcsA or SKC1 (1,2). Recent studies in planar lipid bilayers have shown a pH-dependent gating in KcvNTS. The open probability is decreased from approximately 90% at pH 9 to only 20-30 % at pH 4.

Based on these results, further experiments were performed to analyse the cause of the pH-dependency of KcvNTS. To determine whether the pH-sensing site lies on the intra- or extracellular side of the protein, experiments with pH gradients and dwell time analyses were performed. The data show that the reason for a reduction of the open probability at negative voltages is an additional third closed state, characterised by long closed events and that there are two independent binding sites for H⁺ (or H₃O⁺) block.

Additional experiments were performed with different H⁺- and K⁺-concentrations. The reduction of the open probability due to acidification could be compensated by an increase of the K⁺-concentration. Vice versa, a reduction in K⁺-concentration reduces the open probability already at pH 7. From these results, it can be assumed that H⁺- (or H₃O⁺-) and K⁺-ions are competing against each other to reach the binding sites within the pore. Further, K⁺-ions entering the pore are able to push bound H⁺ (or H₃O⁺) out of the pore.

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P129. Energetics and mechanism of permeation across the formate-nitrite transporters

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Formate-nitrite transporters (FNTs) represent a widely distributed family of membrane proteins that facilitate the transport of small monovalent anions, such as formate and nitrite, across biological membranes. They are structurally similar to the aquaporin water channel, despite the lack of sequence homology. Many aspects of the permeation mechanism of the FNTs are still unknown, such as the nature of proton coupling to the permeation process, as well as the role of a highly conserved histidine residue in the center of the permeation pore.

We use atomistic molecular dynamics simulations to study the permeation across all FNT subfamilies with known structure: the nitrite channel NirC, the formate channel FocA, and the hydrosulfide channel HSC. The energy profiles for permeation of multiple substrates across the FNTs were obtained by potential of mean force calculations. The possibility of a "knock-on" permeation mechanism was studied using computational electrophysiology simulations. Furthermore, we studied the details of the protonation of the central histidine residue. Finally, the substrate protonation during permeation was studied using combined quantum mechanics/molecular mechanics simulations.

These calculations revealed that anions are not able to completely traverse the pore, either due to high free energy barrier for permeation (when the central histidine is neutral), or due to strong binding in the pore (when the central histidine is positively charged). Therefore, the permeating anion needs to be protonated in order to complete the permeation. This process was studied with most details in NirC, for which we propose the following mechanism: in a coupled process, the central histidine is protonated and binds the permeating anion, after which this histidine protonates the anion and enables its release from the binding site in a neutral form.

P130. Towards relating ligand binding and channel gating in nicotinic acetylcholine receptors

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Muscle-type nicotinic acetylcholine receptors (nAChRs) mediate fast synaptic cholinergic responses at neuromuscular junctions. Much is known about the gating behavior of these channels. However, the reciprocal relationship between activation state and agonist binding is still elusive. To contribute to the

better understanding, we applied confocal patch-clamp fluorometry employing a fluorescently tagged ACh derivative, Cy3-ACh, using HEK293 cells expressing adult muscle-type nAChRs.

Cells were lifted after obtaining whole-cell configuration and positioned in front of a double-barreled application pipette. Fast solution exchange was realized by a piezo device. We found a significant difference between desensitization kinetics of cells attached to the chamber bottom compared to lifted cells. (186 ± 21.3 ms versus 85.4 ± 12.8 ms respectively). When Cy3-ACh was applied similarly, we found that Cy3-ACh and untagged ACh is similarly efficient in channel opening ($I_{\max, \text{Cy3-ACh}}/I_{\max, \text{ACh}} = 0.97 \pm 0.01$). Potency for Cy3-ACh ($EC_{50} = 0.91 \mu\text{M}$) was found to be slightly higher than for untagged ACh ($EC_{50} = 2.4 \mu\text{M}$).

For confocal fluorescence imaging, Cy3-ACh was excited with a 543 nm HeNe laser line. To define the cell position for confocal imaging, the background was stained with a reference dye Dy647, excited with a 633 nm HeNe laser line. Application of Cy3-ACh led to fluorescence signals, which were not observed when applied to non-transfected cells. Agonist binding and unbinding appeared as bi-exponential time courses, whereby in both cases the first phase was too fast to be resolved. Interestingly, the second phase of fluorescence increase during agonist application was similar to the time course of desensitization and the second phase of fluorescence decrease during wash-off phase was similar to the time course of recovery from desensitization.

Hereby, we conclude that Cy3-ACh derivative can be a potential tool to study the relation between ligand binding and channel gating.

P131. Unraveling the contribution of each cAMP binding site to HCN channel gating

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HCN channels are cation channels dually gated by hyperpolarization and cyclic nucleotide binding. Cyclic nucleotide binding is mediated through intracellular binding sites located in the C-terminal region of each subunit. Several studies proposed a scenario in which cAMP acts by relieving an autoinhibitory effect of the C-terminal portion of the channel.

To study the contribution of each individual binding step, we used concatenated HCN2 channels with either one, two, three or four disabled binding sites (compare also Ulens and Siegelbaum, Neuron, 2003, 40:959-70). Channel activation following hyperpolarizing voltage jumps was monitored in inside-out macro patches.

We found that constructs with an increasing number of functional binding sites produce a systematic increase in the cAMP-dependent voltage shift of half-maximum activation (one: $DV_{1/2} = 4.2$ mV; two, cis: $DV_{1/2} = 9.9$ mV, two, trans: $DV_{1/2} = 9.5$ mV; three: $DV_{1/2} = 14.3$ mV; four: $DV_{1/2} = 20.9$ mV). The effects of all CNBDs were additive and the position of the two functional binding sites had no impact on the extent of the shift. Interestingly, this additivity was not found when comparing the maximum current amplitude. Only two functional binding sites were sufficient to generate the maximum channel response upon cAMP application. Accordingly, the cAMP-induced deceleration of voltage-dependent deactivation was maximal with only two functional binding sites. However, the speed of voltage-dependent activation was only indirectly determined by cAMP binding through changing the activation level.

In summary, the data obtained herein showed that for a maximum shift of $V_{1/2}$ all four binding sites have to be occupied, suggesting that a full relieve of autoinhibition requires ligand-induced conformational changes in all four subunits. In contrast, other cAMP-dependent parameters as activation and deactivation kinetics do not show this additivity.

P132. Activation of olfactory CNG channels by novel designed nucleotide derivatives

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Cyclic nucleotide-gated (CNG) channels belong to the superfamily of tetrameric voltage-gated channels. CNG channels have a key position for the development of the receptor potential in vision and olfaction. In contrast to classical voltage-gated channels CNG channels are only activated by cyclic nucleotides. In recent studies we used fcGMP in which the cyanine dye DY547 was coupled via an aminoethylthio-linker to the 8'-position of the cyclic nucleotides to analyze the binding and

activation gating in the channels in parallel by confocal patch-clamp fluorometry (Biskup et al., 2007, Nature 446, 440-443). For future single channel experiments a ligand with enhanced affinity and intensified fluorescence quantum yield is desirable.

To achieve this, we first synthesized and characterized a set of novel cGMP and cAMP derivatives. We systematically varied the linker length in the 8'-position of the nucleotides and tested the effects on the activation of the channels. We used the patch-clamp technique in inside-out patches obtained from *Xenopus laevis* oocytes expressing hetero- or homotetrameric CNG channels to determine the apparent affinity of the novel ligands. Another approach is the utilization of a fluorescent dye, which is equipped with a high molecular brightness. To select this dye, a series of different fluorophores was investigated by fluorescence correlation spectroscopy (FCS).

Our results show that a longer aliphatic linker enhances the apparent affinity of a cyclic nucleotide analogue on CNG channels (comparison of EC₅₀ values for the homotetrameric CNG by cGMP with ethyl; hexyl; decyl linker: 1,62; 0,15; 0,08; 0,06 μ M). These results also corresponding in HCN2 channels by cAMP with alkyl chains. With FCS measurements we obtained that coupling of the cyclic nucleotide is strongly affecting the molecular brightness.

In conclusion, there is considerable potential to optimize both the affinity and the brightness of fluorescent cGMP derivatives presently used.

P133. H-bond formation governs the unitary water permeability of narrow channels

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According to classical hydrodynamics, channel geometry determines the unitary osmotic water permeability, pf. This is at odds with the observation that pf of narrow transmembrane channels may vary by several orders of magnitude even though they all are only one water molecule wide and roughly equal in length. We show that the number of poor-lining residues NH which may donate or accept hydrogen bonds determine pf of those channels(1). Water diffusivity depends logarithmic on NH, which is in line with the multiplicity of binding options at higher NH densities. We obtained high-precision pf values by a combination of (i) counting the number of reconstituted aquaporins in large unilamellar vesicles via fluorescence correlation spectroscopy(2, 3) and high-speed atomic force microscopy, and (ii) acquiring the vesicular water efflux from scattered light intensities via our new adaptation of the Rayleigh-Gans- Debye equation(1, 4).

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P134. Regulation and function of two-pore cation channels

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Two-pore channels (TPCs) constitute a family of endo-lysosomal channels with functions in Ca²⁺

signaling and cation homeostasis. Using a mutational approach, we investigated the role of functional domains in TPC1 from *Arabidopsis thaliana*. TPC1 wild type and derived variants were expressed in vacuoles of *tpc1* knockout plants to study their biophysical properties. Thereby, we mapped the sites responsible for vacuolar targeting and activation by calcium, and identified additional regions involved in the regulation of the channel dimer. We furthermore used plant vacuoles as an expression system for mammalian TPC isoforms, in order to reveal similarities and differences in the regulation and function between channel family members of the two kingdoms.

P135. Activation of olfactory CNG channels by newly designed cyclic nucleotide derivatives

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Cyclic nucleotide-gated (CNG) channels belong to the superfamily of tetrameric voltage-gated channels. CNG channels have a key position in the development of the receptor potential in vision and olfaction and are activated solely by the binding of cyclic nucleotides. In recent studies we used the cyanine dye DY547, coupled via an aminoethylthio-linker to the 8'-position of cGMP (fcGMP), to analyze ligand binding and activation gating in parallel by confocal patch-clamp fluorometry. For planned single-channel experiments, ligands with enhanced affinity and optimized fluorescence properties are desirable.

Using inside-out patches from *Xenopus laevis* oocytes we determined the apparent affinity of the novel ligands. First, the native ligands and the corresponding 7-Deaza-purine-bases were tested. With homotetrameric CNGA2 channels, the EC₅₀ values were 52.8 μ M (cAMP), 2.08 μ M (7-Deaza-cAMP), 1.62 μ M (cGMP) and 3.49 μ M (7-Deaza-cGMP). We then systematically varied the linker length in the 8'-position of the nucleotides and tested the effects on channel activation. The results suggest that a longer aliphatic linker enhances the apparent affinity of a cyclic nucleotide analogue: e.g. for homotetrameric CNGA2 channels with cGMP, the EC₅₀ value of 1.62 μ M with cGMP decreases to 0.15, 0.08 and 0.06 μ M with the ethyl, hexyl and decyl linker, respectively.

Another approach is the utilization of a fluorophore with higher molecular brightness than DY547. A plethora of different dyes was investigated in comparison to fcGMP by fluorescence correlation spectroscopy (FCS). Cy3B, DY557 and a Rylene dye were selected and coupled to the cyclic nucleotide by different linkers. With DY557 the photon count per molecule was increased by approximately a factor of two compared to fcGMP.

In conclusion, our results show considerable potential to optimize both the affinity and the brightness of fluorescent cyclic nucleotides presently used.

P136. Shear force activation of the epithelial Na⁺ channel involves the extracellular matrix and N-glycans

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Mechanotransduction describes how cells translate mechanical forces into cellular signals. The epithelial Na⁺-channel (ENaC) formed by α , β and γ subunits is regulated by shear force (SF) and contributes to electrolyte/fluid-homeostasis and blood pressure regulation. The mechanisms how ENaC senses SF are unknown.

Human $\alpha\beta\gamma$ ENaC was expressed in *Xenopus* oocytes and SF-activated currents were recorded with the two-electrode voltage-clamp technique. The contribution of the extracellular matrix (ECM) for SF-activation of ENaC was addressed by degradation of the ECM with hyaluronidase. Site directed mutagenesis was performed to replace asparagines in the extracellular domain of ENaC to reveal their potential function as tethers for SF sensing. Pressure myography was performed to assess the role of the ECM for shear force sensing of ENaC in isolated and intraluminally perfused carotid arteries from mice.

SF-dependent activation of ENaC was observed in *Xenopus* oocytes (increased transmembrane current) as well as in carotid arteries by an augmented vasodilation in response to the ENaC inhibitor amiloride. The SF effects were decreased after hyaluronidase treatment in both *Xenopus* oocytes and arteries, indicating that an intact ECM is required for SF sensation of ENaC. Replacement of N-

glycosylated extracellular asparagines in the α ENaC subunit (N312 and N511) was observed to reduced the SF-activation of ENaC. This indicates that specific N-glycosylated asparagines are involved in SF sensation by providing a connction to the ECM.

Our results uniquely identify the ECM and N-glycans attached to extracellular asparagines as crucial components for SF activation of ENaC. This provides new insights into the mechanism of SF sensation of ENaC suuggesting that the ECM and the N-glycosylated asparagines form/are part of an extracellular tether that connects the channel subunit with the ECM. This could be a new, yet unidentified mechanism for mechanotransduction.

Supramolecular Assemblies and Aggregation

P137. Application of Fast AFM for Studying Dynamic Processes with High Spatiotemporal Resolution

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Atomic force microscopy (AFM) has become a standard for high-resolution structural analysis of samples ranging from single molecules to complex macromolecular systems. A number of new developments in the last years have helped to overcome the limitation of conventional AFM setups, and enabled the analysis of sensitive and dynamic processes, taking place on the millisecond timescale (1, 2). The much faster phase response in phase modulation AFM (PM-AFM) compared to amplitude modulation AFM (AM-AFM), makes possible the application of very small forces and amplitudes of approximately 1-2 Å. We will demonstrate the ease of use of a commercially available PM-AFM setup (HyperDrive™ from JPK Instruments) to achieve submolecular resolution on very soft samples (3), as well as atomic resolution on muscovite mica, simultaneous to fast scanning.

We will discuss examples of applying fast AFM for investigating the dynamics of collagen type I fibrillogenesis, melting of lipid domains, as well as imaging of membrane dynamics & cytoskeleton reorganisation of living cells, demonstrated by realtime videos reaching 1 frame per second. This has been extended to investigating the photomechanics of the 2D protein crystal bacteriorhodopsin, complemented by a nanomechanical characterisation of its trimeric structure (4). We will give examples of the application of a new force tool “Quantitative Imaging” (QI™) based on fast force mapping which offers nanotopographical resolution with the opportunity of simultaneously obtaining a wide range of mechanical properties from different samples.

The full integration of the used tip-scanning AFM setup with commercially available advanced optical microscopes for simultaneous correlative microscopy will be further discussed.

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P138. Computational studies on the structure of amphiphilic dye nanotubes

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Artificial light-harvesting complexes have attracted much interest as candidates for fundamental research on the nature and dynamics of energy transfer and due to their potential applications in the field of opto-electronics.

We are interested in the amphiphilic cyanine dye 3,3'-bis(2-sulfopropyl)-5,5',6,6'-tetrachloro-1,1'-dioctylbenzimidacarbocyanine (C8S3). In aqueous solutions, C8S3 monomers spontaneously self-assemble into long double-walled tubular aggregates with uniform supramolecular structure (nanotubes) [1].

Despite the various experimental data, the resolution of the techniques is not sufficient to reveal atomistic structural details of the C8S3 nanotubes that determine its optical properties [2, 3]. Theoretical models for the molecular arrangement of the C8S3 monomers present good agreement with the experimental spectral properties, but they lack explicit structural information [2, 4].

We performed all-atom and coarse-grained simulations to study the structural and dynamic properties of the C8S3 monomers and nanotubes. At the same time, we compared the performance of different force fields and methods.

Our simulations reveal similarities and discrepancies among the computational results, the experimental structures and the theoretical models, giving insights on the structure of the C8S3 aggregates and ways to improve the current theoretical models.

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P139. Headgroup Interactions Trump Chain Repulsion in Mixed Fluorocarbon/Hydrocarbon Surfactant Micelles

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Surfactants carrying a fluorocarbon chain have become a promising alternative to detergents in membrane-protein research because of their mildness towards fragile membrane proteins (1). However, since hydrocarbons and fluorocarbons are poorly miscible, it is still under debate how fluorosurfactants act in conjunction with conventional, hydrocarbon-based detergents, which are used, for instance, for the extraction of membrane proteins. A particularly intriguing phenomenon is the mixed micellization in binary mixtures of fluorocarbon and hydrocarbon surfactants.

Here, we used a range of experimental approaches to assess the mixed micellization behavior of a fluorinated maltoside surfactant (2) with commonly used hydrocarbon detergents. The latter comprised an alkyl maltoside and a phosphocholine derivative to dissect the influence of headgroup interactions on mixing behavior. We determined critical micellar concentrations for the mixed micelles and applied a non-ideal mixing model based on regular solution theory to quantify the so-called interaction parameter (3). Furthermore, we performed a model-free analysis of the data to derive the composition of mixed micelles even in cases where a single interaction parameter is not suitable (4). From this, we conclude that the repulsion between fluorocarbon and hydrocarbon chains does not necessarily result in demixing of micelles; instead, favorable or unfavorable interactions between the headgroups of the two kinds of surfactants exert a great impact on mixed-micelle formation.

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P140. Diffusion of membrane-bound ligand-receptor bonds

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Protein-mediated membrane adhesion plays a crucial role in a number of biological processes including the immune response and morphogenesis. We aim to understand the effect of lateral interactions between two adhesion bonds on the mobility of the bonds. In the current work, we address this problem by numerical and simulation means. In principle, we find that two adhesive bonds strongly attract each other at short distances and repel at large distances. Naturally such an interaction has a barrier at intermediate separations. We calculate the time it takes for one bond to escape from the attractive well. We also determine the effective diffusion constant of a bond diffusing through an adhesion domain represented by a periodic arrangement of affixed ligand-receptor constructs. This is an important step in understanding the diffusion of bonds, which have recently been measured, and consequently understand the role of the membrane in the process of cell adhesion.

P141. Studying the mechanism of membrane fusion using a synthetic model system

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A system based on two designed peptides namely the cationic (KIAALKE)₃, called peptide K and its anionic complement (EIAALEK)₃, called peptide E has been suggested as a minimal model for studying membrane fusion, inspired by SNARE proteins. It is known that the formation of a dimeric E/K coiled-coil complex causes the docking of vesicles. However, the further process of peptide triggered vesicle fusion could not be explained satisfactorily, yet. Thereby it is of significant interest how the peptides aid to overcome the energetical barriers during lipid rearrangement. Here, investigation of their interactions with neutral PC/PE/cholesterol membranes by several spectroscopic techniques yields insight in the mechanism of membrane fusion using this synthetic coiled coil pair.

P142. Structural polymorphism of enterobacterial lipopolysaccharides in the presence of anti-LPS peptides

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The aggregate structures of bacterial amphiphilic toxins are essential determinants to induce a variety of biological responses in human immune cells, like induction of an inflammation reaction expressed as secretion of tumour-necrosis-factor- α or interleukins by human mononuclear cells (1,2). At high concentrations, these mediators cause a strong inflammation causing the sepsis syndrome (3). It was found that for all Gram-negative endotoxins (lipopolysaccharides, LPS) the existence of a non-lamellar, frequently inverted cubic structure is the prerequisite for the biological responses. These non-lamellar aggregates are able to intercalate into the target cell membranes, disturbing the membrane architecture at the sites of the receptors proteins leading to cell signalling. In contrast, endotoxins with a lamellar periodicity, in particular a multilamellar one, are biologically inactive, because they are unable to induce a membrane disturbance.

In the light of these facts, antimicrobial peptides (AMPs) designed to neutralize sepsis causing toxins need to achieve the conversion of non-lamellar LPS aggregates into a multilamellar structure, to exert a pharmacological action. Here, we present a short summary of a new strategy based on synthetic anti-LPS peptides (SALP) to prevent sepsis.

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P143. Phase equilibria in lipid nanodiscs

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Nanodiscs formed by phospholipids and styrene maleic acid (SMA) copolymers are gaining growing attention as membrane mimetics for membrane-protein research (1). To a first, crude approximation, such SMA/lipid particles (SMALPs) are envisaged to be around 10 nm in diameter and to comprise a lipid-bilayer core surrounded by a thin polymer rim. In reality, however, things are more complex, as both the size and the composition of SMALPs vary with the types and the concentrations of both lipid and polymer components (1, 2).

Here, we present a quantitative approach to relate SMALP size and the compositions of its core and rim to the concentrations of lipid and polymer species. This model is an extension of the pseudophase concept frequently applied to canonical lipid/detergent systems (2), as it treats the core and the rim of a nanodisc as distinct pseudophases. In combination with dynamic light scattering, calorimetry, and ³¹P NMR data, this model provides a detailed structural picture of nanodiscs. In particular, and in contrast with previous conclusions, it turns out that virtually all polymer is associated with the nanodisc, thus accounting for a substantial fraction of its total volume, where a considerable amount of polymer is found in the bilayer core of the SMALP. These findings contribute to a better understanding of nanodisc structures, the underlying thermodynamics of SMALP formation, and their potential and limitations as membrane mimetics for studying membrane proteins and lipids.

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P144. Molecular factors responsible for the modulation of the lipid-induced aggregation of α -synuclein

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The conversion of soluble monomeric alpha-synuclein into amyloid fibrils is the hallmark of a range of neurological disorders, including Parkinson's disease. Unlike other amyloidogenic proteins, such as the A beta peptide, involved in Alzheimer's disease, α -synuclein is surprisingly stable in solution under quiescent conditions and its aggregation is mainly triggered via interaction with surfaces including air/water interfaces, hydrophobic polymers, surfactants, and lipid membranes.

Here, I will present our recent findings which provide a rationale for the observed modulation of the kinetics of α -synuclein aggregation by model membranes. In particular, we have recently shown that negatively charged lipid vesicles can trigger the aggregation of α -synuclein by enhancing the rate of primary nucleation by up to 3 orders of magnitude. In addition, our results indicate that both the protein:lipid ratio and the chemical properties of the lipids play an important role in modulating the kinetics of the lipid-induced aggregation of α -synuclein.

These findings are essential for elucidating the molecular determinants responsible for the switch between functional and deleterious interactions between α -synuclein and membranes.

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P145. The curious case of phosphoketolase - a huge crystallographic unit cell filled without symmetry

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Phosphoketolase (PK) is a thiamin-dependent enzyme central to the energy metabolism of certain bacteria. PK's reaction is an interesting hybrid of two other well-studied thiamin-dependent enzymes: it catalyzes the cleavage of sugars, like transketolase, and it produces acetylphosphate, like pyruvate oxidase. The two steps are linked by a dehydration reaction that is unique among the thiamin-dependent enzymes.

To study the catalytic machinery, PK from *Lactobacillus pentosus* (LpPK) was crystallized. X-ray diffraction yielded a very curious result: the crystal's unit cell was stunningly large at 8500 nm³ and, worse, had no internal symmetry at all: space group P1, despite some tempting peaks in the self-rotation function. LpPK is rather big (788 aa), but it would still take 25-45 independent copies to fill this kind of cell. Ultimately, with a resolution limit of 3.6 Å in our best dataset, structure determination was deemed impossible.

Then, in 2010, high-resolution structures of two related PKs were published, derived from crystals with friendlier properties.[1,2] Although the enzymological questions could now be answered, we were still curious about the content of "our" unit cell. We succeeded by employing the new structures for a molecular replacement solution of the data set: our cell contains 36 copies of LpPK, stacked in three rings. The stacking axis is almost - but not quite! - parallel to a unit cell edge. In each ring, six LpPK dimers are wedged around a central hole, with one gap between two of the dimers. These gaps align in adjacent rings. The resulting arrangement in the crystal resembles a bundle of drinking straws that have been sliced open; it looks surprisingly regular, yet has no true crystallographic symmetry.

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Membrane Architecture

P146. Poly-arginines and free energies of trans-membrane pore formation

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Trans-membrane pores are transient structures that play an important role in translocation of small molecules and peptides across cell membranes. Cell-penetrating and anti-microbial peptides act via pore-mediated mechanisms. Characterizing the free energy landscape of pore-formation is crucial for designing antimicrobial peptides. However, the free energy landscape of pore formation remains poorly understood, partly because potential of mean force (PMF) calculations of pore formation strongly depend on the choice of the reaction coordinate. We used umbrella sampling to compute PMFs for pore formation using three different reaction coordinates, namely, (i) a coordinate that steers the lipids in the lateral direction away from the pore center, (ii) the distance of a single lipid phosphate group from the membrane center, and (iii) the average water density inside a membrane-spanning cylinder. Our results show that the three reaction coordinates suffer from strong hysteresis between pore-opening and pore-closing simulations, suggesting that they do not restrain the systems close to the transition state for pore formation. Further analysis suggests that the formation and disruption of a continuous hydrogen-bonding network across the membrane corresponds to the transition state for pore formation [1]. Finally, using an optimal reaction coordinate, we report free energies of pore formation for different lipids in the absence and presence of poly-arginine fragments. Our atomistic simulations provide quantitative comparison between pore-formation in anionic and zwitterionic lipids. We find that poly-arginines alter the equilibrium membrane structure, and hence, influence pore nucleation. For anionic lipids, the concentration and electrostatic charges of poly-arginine fragments play a crucial role. Our simulations provide atomistic and energetic insight into the mechanism underlying pore formation in lipid membranes.

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P147. A Comparative Study on Techniques for the Determination of Lipid Bilayer Permeabilization

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Host defense peptides (HDPs) are small effector molecules of the innate immune system building up the first defense barrier when it comes to infections. Infectious diseases are commonly treated with antibiotics leading to an increase in antibiotic resistances among clinically relevant pathogens. Therefore, understanding the mode of function of alternative agents with antibiotic function is undeniable for the successful development of new antibiotics opening new treatment options. To understand recognition-, recruiting- and permeabilization-processes on membrane surfaces a set of structurally diverse peptides is investigated regarding their membrane active function on charged and uncharged model membranes. Lipid bilayers are reconstituted from pure DOPC to mimic the inner leaflet of the bactericidal cell envelope with an overall negative surface charge. Permeabilization events induced by the antimicrobial peptides LL-32, Arenicin-1 and hBD-3-I as well as the ionophore Nonactin and α -Hemolysin of *Staphylococcus aureus* are investigated with respect to the membrane systems stated above and buffer compositions (high-salt vs. low-salt). A combination of a complex array of biophysical techniques is taken into account for studying permeabilization events with reducing limitations to a minimum – MONTAL-MUELLER-Setup for planar lipid bilayers, tethered membrane technology for supported bilayers, liposome-based assays (Calcein release assay, potassium iodide quenching, FRET-intercalation assay) and a chip-based pore spanning dye release assay. These methods allow to investigate the whole spectrum ranging from single pore characterization to the statistical analysis of either large membrane areas or a huge number of single membranes.

P148. Mechanical properties of giant vesicles isolated from the plasma membrane

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Giant Unilamellar Vesicles are a popular membrane model because of their accessibility by many experimental methods. While the ability to precisely control the composition of such membranes is advantageous, reconstitution of membrane proteins in GUVs suffers from some limitations. Only systems of low compositional complexity, low protein densities and random protein orientation in the bilayer can be obtained. In contrast, the plasma membrane of the living cell is highly crowded, its composition is complex and proteins have a defined orientation. As a missing link between these two extremes, chemically lysed plasma membrane vesicles have recently attracted much renewed interest. These vesicles, also called giant plasma membrane vesicles (GPMVs), have attracted attention because of their ability to exhibit optically resolvable liquid-liquid separated domains which are inhibited in the plasma membrane (1). Recently, we have demonstrated the use of GPMVs to reconstitute membrane proteins and study their function in a near native environment (2). In this work, we study the mechanical properties of GPMVs by fluctuation analysis and micropipette aspiration. We compare how various isolation conditions, such as chemical composition of solutions used to derive GPMVs, cell densities and cholesterol depletion, influence the bending rigidity. Furthermore, we measure the GPMV bending rigidity as a function of temperature and assess their phase separation. With these measurements, we hope to contribute to the understanding of the mechanical properties of the native plasma membrane.

This work is part of the MaxSynBio consortium which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society.

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P149. Functional solubilization of a membrane protein into new lipid/polymer nanodiscs

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Once removed from their natural environment, membrane proteins depend on self-assembling membrane-mimetic systems to retain their native structures and functions. To this end, lipid-bilayer nanodiscs that are stabilized by membrane scaffold proteins (MSPs) or amphiphilic polymers are becoming increasingly popular as mild alternatives to conventional detergents for in vitro membrane-protein research. The recent use of styrene/maleic acid (SMA) copolymers is particularly appealing because they extract proteins directly from membranes.

Here, we show that an alternating diisobutylene/maleic acid (DIBMA) copolymer performs on par with or better than SMA in solubilizing phospholipids and membrane proteins into functional bilayer nanodiscs. Upon addition to proteoliposomes, DIBMA efficiently forms chemically and thermally stable nanodiscs that have a narrow size distribution and support the activity of an integral membrane enzyme. Unlike MSPs and SMA, DIBMA has a mild impact on lipid chain order and is compatible with optical spectroscopy in the far ultraviolet wavelength range without removal of empty nanodiscs, which offers substantial advantages for in vitro studies on integral membrane proteins.

P150. Influence of lipid bilayer properties on nanodisc formation driven by styrene/maleic acid copolymers

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Styrene/maleic acid (SMA) copolymers are attracting great attention as mild but powerful alternatives

to detergents for the extraction and stabilization of membrane proteins. Whereas structural aspects of “native nanodiscs” resulting from polymer treatment of biological and model membranes have been characterized in detail, the underlying mechanism of nanoparticle formation remains poorly understood. We report herein the thermodynamics of solubilization of vesicles composed of saturated or unsaturated phospholipids by SMA copolymers at different temperatures. We systematically investigated the aggregational state of phospholipids upon exposure to SMA having a 3:1 styrene/maleic acid molar ratio with the aid of ^{31}P NMR spectroscopy and dynamic light scattering. The results show that the solubilization process can be conceptually rationalized in terms of a pseudophase model originally developed for detergent/lipid interactions. The solubilizing power of SMA(3:1) towards a saturated lipid is higher in the gel than in the liquid-crystalline state of the membrane, although solubilization proceeds more slowly. On the basis of the phase diagrams obtained, re-association of polymer-solubilized lipids with vesicles is possible under mild conditions, which has implications for the reconstitution of proteins and lipids from nanodiscs into vesicular membranes.

P151. The distribution of C-type lectin receptors in the Dendritic cell membrane

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Dendritic cells (DCs) are the most important antigen-presenting cells in the immune system. They are responsible for the recognition and internalization of pathogens, followed by the induction of antigen-specific CD4⁺ and CD8⁺ T cell responses, but also for the maintenance of peripheral T cell tolerance. DCs express a variety of endocytic receptors such as C-type lectin receptors (CLRs), which are important for the defense against invading pathogens. CLRs are able to bind and internalize a variety of different pathogens, which can then lead to DC maturation. These receptors are potential candidates for immunomodulatory therapeutic intervention as the delivery of chimeric antigen targeting antibodies to such receptors (e.g. DEC205 or DCIR) induced strong CD4⁺ and CD8⁺ T cell responses (Dudziak et al., 2007, Neubert et al., JI, 2014).

Here we want to further investigate the distinct antigen uptake and presentation properties of the most common antigen targeting receptors DEC205 and DCIR. In multi-color confocal microscopy (up to 6 different fluorochromes) of human monocyte derived DCs (moDC) we found that DEC205 and DCIR demonstrated a distinct and mutually exclusive distribution in the membrane. This expression pattern might indicate that recognition and uptake of pathogenic material into endosomal compartments might not only be directed by the endocytic receptor itself, but also by the localization of the receptor in the membrane of a phagocytic active cell. To further elucidate if this spatial distribution might be valid also for other CLRs we have tested the macrophage mannose receptor MMR as well as DC-SIGN and found that MMR showed a similar profile than DCIR while DC-SIGN was only partially overlapping with DEC205, but not with DCIR or MMR. In order to obtain a more detailed picture of the receptor localizations we are establishing high resolution microscopy using STimulated Emission Depletion (STED).

P152. Solid supported model membranes from plant lipids and their interactions with long-chain polyamines

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Diatoms are protected by a silica cell wall produced in silica deposition vesicles. The structure of the cell wall is presumably controlled by aggregation of different biomolecules, including silaffins, cingulins and long-chain polyamines (LCPAs), which act as a template for silica deposition.(1) As silica biomineralization takes place in a membrane enclosed compartment we investigate the interactions of the previously mentioned biomolecules and lipid bilayers.

Diatom membranes are mainly composed of the glycolipids MGDG, DGDG and SQDG as well as PG and PC.(2) We accomplished to generate and characterize model membranes on solid supports closely reflecting the overall lipid composition in diatoms. Both supported lipid bi- and monolayers have been prepared by spreading of small unilamellar vesicles on hydrophilic mica or hydrophobic

functionalized surfaces.

The LCPAs are linear molecules with repeating amine functions which catalyze precipitation of silica from solution.[3] We investigate the interactions of a synthetic polyamine[4] with our model membranes. Addition of LCPAs to lipid membranes leads to the formation of micrometer-sized domains. These domains were analyzed by atomic force and fluorescence microscopy. Height differences of about 8 nm or 15 nm between the domains and their surroundings as well as increased fluorescence intensities of the factor 2.0 ± 0.2 or 3.1 ± 0.4 revealed the presence of membrane stacks with one or two additional lipid bilayers on top of a continuous underlying one. A connection between both the underlying and stacked membranes was proven by fluorescence recovery after photobleaching showing the diffusion of lipids from the surrounding membrane into all layers of the stacks.

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P153. In silico study of miltefosine and its influence on membrane properties

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Miltefosine is a small alkyllysophospholipid analogue, which possesses anti-neoplastic and even anti-tumor activities. Even though its structure is simple, consisting of a zwitterionic head group and one saturated fatty acid tail with sixteen carbons, miltefosine is reported to trigger apoptosis in cancer cells and various parasites. Hence it became the first and still only oral drug to treat Leishmaniasis, a disease induced by parasites of genus *Leishmania* and transmitted through the bite of a sandfly.

Despite the fact that miltefosine rather targets cellular membranes than the DNA, the mechanism of action is still not fully understood. Recent studies propose that miltefosine modulates the membrane permeability, phospholipid turnover, and signal transduction in cell membranes of the parasite [1]. The disturbance of phospholipid metabolism caused by miltefosine was reported to result in cell death [2].

In this study, we explore the influence of miltefosine on membrane properties. Using coarse-grained and all-atom simulations we analyzed dynamic differences in phospholipid bilayers with and without miltefosine.

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P154. Lipid transfer from supported lipid bilayer to cortical neurons

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Supported lipid bilayer (SLB) has been used widely as a model membrane because it is easy to observe and retains the characteristics of cell membrane.[1, 2] Cell membrane works not only as a barrier, but also works to transport materials and even exchange membrane components as a mean of cell-cell communication.[3] But, direct observation of intercellular membrane transfer is still technically challenging. Here, we show that SLB can show the lipid transfer from model cell membrane to cortical neurons cultured on positively charged SLB.

Positively charged lipid is essential in SLB for neurons to adhere.[4, 5] Rat primary cortical neurons were cultured on SLB which is made by lipid vesicle deposition on glass surface. Fluorescence labeled lipid molecule is inserted in the SLB to observe the movement of lipid molecules. At 6th day of culture, neurons on highly positive (75% and above) SLB showed fluorescence from their plasma membrane. Neurons also proliferate on the SLB with less charge, but their plasma membrane did not show any fluorescence. After day 6, fluorescence of lipids was still observed from dead cells, but not from live cells which shows neurons can remove transferred lipids and renew its membrane after 1 week. These results show that lipid can diffuse to plasma membrane, but biological mechanism also exist for highly

charged SLB.

Lateral mobility and chemical similarity has been the main characteristics of SLB to be used as a model membrane. As shown here, lipid and membrane transfer is also observable by culturing cells directly on SLB.

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Cell Biophysics, Intracellular Transport, and Signalling

P155. Topological Dynamics, Phase Transitions, and Foraging of *Physarum polycephalum* transport networks

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The slime mold *Physarum polycephalum* is a unicellular but multi-nucleated organism, which amazingly can grow up to square meters in size. It forms extended transport networks in order to search for food. These networks exhibit a rich oscillatory dynamics on various length and time scales. The structure of the foraging units is dependent on environmental conditions and life stage. We find oscillating microplasmodia, which percolate into a network directly [1] or fuse into compact satellites before transforming into networks as well. We model the percolations transition, which occurs in well-fed conditions, analytically within the configuration model of graph theory utilizing all partaking types of nodes. Quite generally, we find that at the percolation transition the formation of a small link degree network is topologically highly constrained and only weakly dependent on environmental factors. Structuring of the network as characterized by global efficiency and centrality measures precedes area search in order to forage [2]. In contrast, satellites are found to be predominant after prolonged starvation of microplasmodia. The number and size of satellites forming out of a spherical patch of single microplasmodia shows characteristic scaling behavior with coverage. After radially swarming away from the patch, satellites develop holes and transform into networks as well. We have obtained flow patterns within external (network) and internal (satellite) veins and gained ultra-structural insights into their morphology and topology. Global network topology is modeled with a non-linear master equation for the link degree distribution. The adaptive networks of *Physarum* are capable of hydrodynamic computing and may serve as non-neuronal models for universal mechanisms of decision-making [3].

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P156. Formation of epithelial tissues: mechanosensitivity and space tessellation on micro and macro scale

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While it is well established that the mechanosensitivity determines the structure of the cytoskeleton, and the protein expression in single cells, the coupling between mechanical and biochemical signals in tissues remains a challenging issue. We address this problem in model epithelium, grown from MDCK II cells on substrates with various rigidities. We study the formation of the monolayer as a function of the of the elastic stiffness of the underlying polyacrylamide substrates coated with collagen I. At physiologically relevant, soft substrates (0.6 ± 0.2 kPa), a monolayer with a very high, steady cell density, surrounded by a three-dimensional unstructured compartment of diving cells, is found already in clusters of 0.005 mm^2 [1]. The density in this novel regime of growth was found to be independent of time and cluster size. On hard substrates (elasticity > 3 kPa), where the commonly reported growth was found, the tissue compartmentalizes over several days, after reaching millimeter sizes. Thereby a contact inhibited bulk region, followed by a proliferating compartment and a low density moving edge could be distinguished. We model formation of the bulk and edge region, based on Voronoi tessellation, within which we are able to simulate the growth and the associated compartmentalization, thereby providing deeper understanding of the observed phenomena. Furthermore, we develop a new tessellation method based on the shape of the cells' nuclei and prove that our method is systematically better than the existing Voronoi tessellation for all morphological measures. Success of this method implies membrane-nucleus coupling that was widely debated in single-cell mechano-transduction.

Equilibration of stress between neighboring cells occurs simultaneously with the equilibration of internal stress on the nuclei, which in turn translate into biochemical signals promoting or inhibiting proliferation and migration.

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P157. Co-localization Studies of Ror2 with membrane microdomains

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Receptor tyrosine kinase-like orphan receptor (Ror) proteins are a conserved family of tyrosine-kinase receptors that function in developmental processes including skeletal and neuronal development, cell movement and cell polarity. Ror proteins are type I single-transmembrane domain proteins and act as Wnt co-receptors (1). Ror2, one of the isoform of Ror proteins plays a pivotal role in the Beta-Catenin-independent non-canonical Wnt pathways. The ability of Ror2 to activate non-canonical Wnt signaling occurs after Clathrin-mediated endocytosis and Ror2 interacts also with Caveolin at the plasma membrane (2).

We hypothesize that Ror2 associates with different membrane microdomains, can shuttle between different membrane microdomains and different localization affects signaling. The length and amino acid sequence of the transmembrane domain (TM) and its membrane proximal regions are crucial for the interaction of membrane proteins with lipid microdomains. Colocalization analysis was used to identify Ror2 domain(s) that mediates interaction with specific microdomains. The values of co-localization were estimated using JACoP, a tool which is enable to calculates different parameters such as Manders coefficient, overlap coefficient and Costes automatic threshold methods. Based on these calculations, around 55% of WT-Ror2 and Ror2_310-422 was found in areas associated with Caveolin (Cav1). Around 50% of WT-Ror2 and Ror2_310-469 were also found associated with Flotilin (Flo2). These results indicate that not only the TM domain of Ror2 but also its proximal regions might be involved in mediating the interaction with distinct membrane domains.

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P158. N-glycosylation enables lateral mobility of GPI-anchored proteins at a molecular crowding threshold

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The protein density in biological membranes can be extraordinarily high, but the impact of molecular crowding on the diffusion of membrane proteins has not been studied systematically in a natural system. The diversity of the membrane proteome of most cells may preclude such systematic studies on individual proteins. African trypanosomes, however, feature a uniform surface coat that is dominated by a single type of variant surface glycoprotein (VSG). Here we study the density-dependence of the diffusion of different GPI-anchored VSG-types on living cells and in artificial membranes. Our results suggest that a specific molecular crowding threshold (MCT) exists that limits diffusion and hence affects protein function. Obstacles in the form of heterologous proteins compromise the diffusion coefficient and the MCT. The trypanosome VSG-coat operates very close to its MCT. Importantly, our experiments show that N-linked glycans act as molecular insulators that reduce retarding intermolecular interactions allowing proteins in biological membranes to function correctly even when densely packed.

P159. Predicting bacterial growth transitions without kinetic parameters

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A grand challenge of systems biology is to predict the kinetic responses of living systems to perturbations starting from the underlying molecular interactions. Changes of the nutrient environment have long been used to study regulation and adaptation phenomena in microorganisms, and remain a topic of active investigation in the era of systems biology. In these studies, the challenge of understanding how a bacterium adapts its growth after a nutrient change lies in deciphering the regulation of its gene expression in response to the applied perturbation. While much is known about the molecular interactions governing the regulation of key metabolic processes, they remain insufficiently quantified for predictive bottom-up modeling. Here we develop a top-down approach, expanding recently established coarse-grained proteome allocation models, from steady-state growth into the kinetic regime. Using only qualitative knowledge of the underlying regulatory processes, we derive a quantitative model of bacterial growth transitions which accurately predicts the time course of gene expression and biomass accumulation in response to both nutrient up- and down- shifts (e.g., diauxic shifts) without kinetic parameters. The results reveal a simple adaptation strategy comprised of an initial phase of maximal synthesis of new proteome components, followed by a slow dilution of the inherited proteome until the final composition is reached. More generally, the ability to accurately predict bacterial adaptation with coarse-grained models suggests a path for understanding a broad range of adaptive processes without detailed characterization of the underlying biochemical reactions.

P160. Label free in vitro measurement of cell-chip distance using surface plasmon resonance microscopy

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Longterm investigation of neuronal networks require non-invasive recordings of the electrical signals. A good coupling between the biological and electronic system is crucial and depends particularly upon the cell-chip distance. The cell-chip distance is an important parameter towards a good sealing, with closer contact leading to a decreased signal dissipation in the cell-electrode cleft. We therefore try to optimize the contact geometry of said interface using protein and lipid coatings. In order to measure the distances between the cell membrane and the chip surface in vitro, we built a surface plasmon resonance microscope (SPRM). [1]

With gold coated sapphire chips as the substrate for the cell culture, it is possible to excite plasmons (collective electron oscillations) in the gold layer by illuminating it under a specific angle. The resonance frequency of the plasmons depends strongly upon the dielectric constant of the gold's environment. In turn the angle spectrum of the reflected light depends upon said resonance frequencies. Due to these dependencies it is possible to deduce the cell-substrate distance.

Our microscope is capable of imaging the interface in two different modes. The field of view in the live imaging mode is around 65 μm x 65 μm . This is useful for determining the region of interest for the scanning mode. This mode uses localized surface plasmons to measure the cell-substrate distance. The resolution in z-direction lies in the nanometer range. This allows us to accurately characterize the cell-chip interface.

Since SPRM is non-invasive and label free it is suited for longterm investigations.

It is therefore possible to observe the development of neuronal networks over several weeks.

Label-Free Measurement of Cell–Electrode Cleft Gap Distance with High Spatial Resolution Surface Plasmon Microscopy. Koji Toma, Hiroshi Kano, and Andreas Offenhäusser. ACS Nano 2014 8 (12), 12612-12619

P161. Optical recording of voltage-signaling events in sperm

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In human sperm, activation of CatSper Ca²⁺ channels by the female sex hormone progesterone evokes a rapid biphasic Ca²⁺ response. It has been proposed that Slo3, the principal K⁺ channel in sperm, mediates a Ca²⁺-induced hyperpolarization that shuts off CatSper and, thereby, shapes the Ca²⁺ response. To scrutinize this notion, we established optical recordings of progesterone-induced voltage responses in human sperm. To this end, we utilized a novel class of fluorescent voltage indicators: voltage-sensitive photo-induced electron transfer (PeT) dyes. We show that PeT dyes faithfully report the membrane potential (V_m) of sperm. Using sea urchin sperm as a model, we characterized a series of voltage-sensitive dyes and demonstrate that the PeT dyes BeRST and VF2.1Cl outperform the widely-used electrochromic voltage indicators. Moreover, BeRST is compatible for multiplexing with fluorescent indicators of Ca²⁺ and pH, which enabled simultaneous recording of multiple signaling modalities in sea urchin sperm. Finally, we utilized VF2.1Cl to monitor changes in membrane potential. We revealed that progesterone evokes multiphasic voltage responses, supporting the hypothesis of a CatSper-Slo3 interplay during progesterone signaling.