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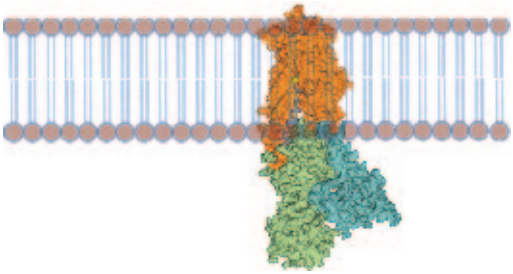
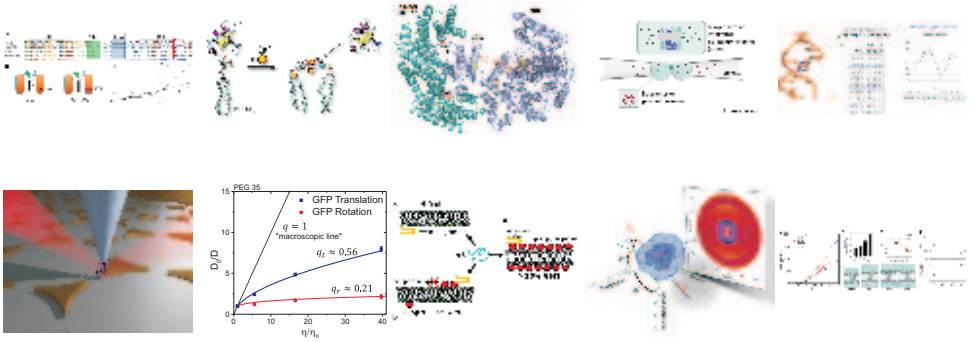


Joint Meeting of Czech and German Biophysicists

Structural Transitions of Biomolecules in Experiment and Theory



ABSTRACT BOOK

23rd-25th February 2017

Monastery "Kloster Hünfeld", Germany

ACKNOWLEDGEMENTS

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CONFERENCE SCHEDULE

Thursday Feb. 23rd

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14:40 - 15:10	Schröder, Indra *K^+ channel selectivity filter gating correlates with site-specific ion occupation obtained from the same experiment*
15:10 - 15:30	Elicharová, Hana (selected talk) *Potassium uptake systems in Candida species*
15:30 - 16:00	Coffee Break
16:00 - 16:30	Zayats, Vasilina *Modulation of human ORAI1 channel: modeling and simulations*
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2.) Protein folding and protein structural substate analysis

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17:20 - 17:50	Bednar, David *FireProt: Computational Design of Thermostable Proteins*
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19:00 - 19:30	Schulze, Andrea (selected talk) *Cooperation of Local Motions in the Hsp90 Molecular Chaperone ATPase Mechanism*

CONFERENCE SCHEDULE

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3.) Membrane receptors and their dynamic interactions with lipids

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9:30 - 10:00 Böckmann, Rainer
GPCR dimerization and activation studied in sequential multiscale simulations

10:00 - 10:30 Schneider, Bohdan
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4.) New techniques A

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13:50 - 14:20	Coskun, Unal *Calcium directly regulates phosphatidylinositol 4,5-bisphosphate headgroup conformation and recognition*
14:20 - 14:40	Martinez-Seara, Hector (selected talk) *Determinants of sodium and calcium adsorption onto neutral lipid bilayers and how the cell can use them to modulate hyaluronan-membrane interaction*
14:40 - 15:10	Amaro, Mariana *Impact of GM1 nanodomains in the oligomerisation of membrane bound Aß monomers*
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16:00 - 16:30	Vachá, Robert *Anomalous Interactions of Amyloids with Surfaces*
16:30 - 16:50	Hajiraissi, Roozbeh (selected talk) *Adsorption and aggregation of hIAPP at different self-assembled monolayers*
16:50 - 17:20	Cwiklik, Lukasz *Tear Film Lipid Layer: a molecular-level view*
17:20 - 17:50	Jungwirth, Pavel *Cell Penetration and Membrane Fusion: Two Sides of the Same Coin*
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13:00 - 13:30 Havenith, Martina
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CONFERENCE SCHEDULE

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15:00 – 15:20	Božíková, Paulína (selected talk) *Annotation of DNA Structures by a Newly Formulated DNA Structural Alphabet*
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17:00 - 17:20	Höfig, Henning (selected talk) *Single-Molecule Studies on CFP-YFP-based biosensors*
17:20 - 17:40	Schwieger, Christian (selected talk) *Infrared Reflection Absorption Spectroscopy: a Potent Method to Study Membrane Binding (Macro)molecules*
17:40-18:00	Rudack, Till (selected talk) *From atom to cell: Integrating experimental results and user expertise into computational modeling*
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P-3	*Energetics and mechanism of permeation across the Formate-Nitrite Transporters (FNTs)* *(selected talk)*	Atkovska, Kalina
P-4	*Trans-membrane pore formation: free energies and reaction coordinates*	Awasthi, Neha
P-5	*Calcium directly regulates phosphatidylinositol 4,5-bisphosphate headgroup conformation and recognition*	Bilkova, Eva
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P-8	*Potassium uptake systems in Candida species* *(selected talk)*	Elicharová, Hana
P-9	*Membrane interaction and conformational changes of α-synuclein* *(selected talk)*	Fallah, Mohammad A.
P-10	*Membrane Lateral Pressure Regulates Dipolar Relaxation Dynamics at the Active Site of an ATPase*	Fahmy, Karim
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P-18	*Determinants of sodium and calcium adsorption onto neutral lipid bilayers and how the cell can use them to modulate hyaluronanmembrane interaction (selected talk)*	Martinez-Seara, Hector
P-19	*Time-domain THz spectroscopy reveals coupled protein-hydration dielectric response in solutions of native and fibrils of human lysozyme (selected talk)*	Novelli, Fabio
P-20	*Solvent effects of H_2O and D_2O on the site-directed fluorescently-labeled P_{IB}-type ATPase CopA from Legionella pneumophila*	Nucke, Lisa
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P-28	*Conformational changes of Channelrhodopsin-2 investigated by time-resolved EPR spectroscopy*	Schumacher, Magdalena
P-29	*Infrared Reflection Absorption Spectroscopy: a Potent Method to Study Membrane Binding (Macro)molecules (selected talk)*	Schwieger, Christian
P-30	*Lipid nanodiscs with genetically engineered MSP1D1 for structural and functional studies (selected talk)*	Subramanian, Madhumalar
P-31	*Differential Ca^{2+}-sensing by GCAPs in rod and cone cells provide molecular basis of step-by-step regulation of retinal guanylate cyclase upon light activation (selected talk)*	Sulmann, Stefan
P-32	*Dissecting steps in ATP-driven protein translocation through the SecY translocon*	Tuma, Roman
P-33	*Interaction between HDAC6 and tubulin dimers/ microtubules*	Ustinova, Ksenya
P-34	*Diisobutylene/maleic acid copolymer solubilizes membrane proteins into functional lipid-bilayer nanodiscs*	Vargas, Carolyn
P-35	*K^+ channel selectivity filter gating correlates with site-specific ion occupation obtained from the same experiment*	Schroeder, Indra
P-36	*SOD1 as a novel sensor for in-cell protein folding experiments*	Ahlers, Jonas

TALK ABSTRACTS

Yeast as a tool to study cell membrane transporters

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Yeast *Saccharomyces cerevisiae* is one of the most thoroughly researched eukaryotic microorganisms. Besides sharing the complex internal cell structure of plants and animals, it is mainly its fast growth, a broad choice of genetic tools to add new genes or delete its own genes through homologous recombination, and the complete sequencing and annotation of its genome as the first one amongst eukaryotic cells, which make it highly interesting to study cell transport processes. Importantly, the broad use of yeast cells in research is also driven by biotechnological applications with a growing economic impact. *S. cerevisiae* has been used to characterize structure and function of broad variety of eukaryotic transporters including plant and mammalian systems for influx and efflux of alkali metal cations. We will present examples how the construction of yeast mutants lacking cation transporters helps in a) identification of amino-acid residues involved in cation selectivity of Na^+/H^+ antiporters [1], b) in establishing a system for HTS of putative modulators of mammalian potassium channel [2], and c) in identification of new molecular targets for development of antifungal drugs [3.

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Modulation of human ORAI1 channel: modeling and simulations

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Orai1 is a calcium-selective channel located in the plasma membrane, belonging to the family of calcium-release activated channels (CRAC) [1]. Orai1 is a component of store-operated calcium entry (SOCE) that is activated by STIM1, the second component of SOCE, when intracellular calcium stores are emptied. STIM1, located at the endoplasmatic reticulum (ER), senses levels of calcium in ER and is activated by calcium store depletion. In turn, calcium influx via the Orai1 channel refills calcium levels in the ER [2]. The crystal structure of the *Drosophila melanogaster* Orai channel reveals a hexameric assembly of the protein with a single monomer of ~250 amino acids folded into four transmembrane helices [3]. Based on the Orai crystal structure a homology model of the human Orai1 channel was prepared that includes extracellular and intracellular loops existing only in human isoform [4]. The sequence and architecture of the Orai channels is unique among other ion channels and suggests a novel gating mechanism. This study focuses on the central ion pore to investigate the gating mechanism of this unique channel. The pore starts from the selectivity filter, formed by the ring of six glutamate residues, further down the pore followed by hydrophobic region and basic region toward the intracellular side. Using combined experimental and theoretical approaches of structure-guided mutagenesis, patch-clamp, cysteine-crosslinking and molecular modeling, the ion pore of the human Orai1 channel was investigated. Mutations within the Orai1 channel affecting its gating were linked to immune diseases and cancer [5]. To investigate a gating mechanism several Orai1 mutants occuring in tumor cells and able to switch the channel from constitutively open to an inactive state were studied. Using molecular modeling structural or dynamical changes within the pore caused by those mutations were observed and analysed on an atomic level.

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Biomolecular dynamics studied with residue-specific resolution

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Protein folding involves several structural transitions including backbone ordering, hydrogen bond formation and side-chains packing. These fundamental processes are difficult to observe with equilibrium measurements and are better analyzed with perturbation techniques. Pulsed laser-excitation can be used to generate fast jumps in temperature or in pH and to study non-equilibrium relaxation dynamics of peptides and proteins. We built-up a quantum cascade laser based IR-spectrometer combined with a laser-excited temperature-jump (T-jump) or alternatively with a pH-jump for the fast (nanosecond) initiation of conformational dynamics. Peptide models and non-perturbing site-specific probes were used to study folding mechanisms at the level of individual amino acids. In T-jump experiments, we analyzed the relative importance of turn versus intrastrand stability in β-sheet formation [1] as well as the effect of hydrophobic interactions [2]. In a pH-jump study, we investigated the coiled-coil formation of a pH-sensitive leucine zipper peptide beyond the diffusion limit [3]. Both perturbation techniques were applied to poly-L-glutamic acid (PGA) in order to induce folding (pH-jump) and unfolding (T-jump) of PGA. We quantified the increase respectively decrease in helicity. Two kinetic steps were observed for both, pH-jump and T-jump induced dynamics indicating the same molecular mechanism for helix folding/unfolding irrespective of the perturbation technique used [4].

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FireProt: Computational Design of Thermostable Proteins

David Bednar[1,2], Milos Musil[1,2,3], Koen Beerens[1], Eva Sebestova[1], Jaroslav Bendl[1,2,3], Radka Chaloupkova[1], Zbynek Prokop[1,2], Jan Brezovsky[1], Jaroslav Zendulka[3], Jiri Damborsky[1,2]

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Stability is an important property enhancing enzymes utility as biocatalysts, therapeutics, diagnostics, and nanomaterials. In comparison with experimentally very demanding directed evolution, computational methods offer a fast and inexpensive alternative to protein stabilization. However, stability prediction tools have only a limited reliability due to potentially antagonistic effects of substitutions. Therefore, only single-point mutations are usually predicted in silico, experimentally verified and then recombined into multiple-point mutants. Here we present the computational method FireProt, combining energy- and evolution-based approaches for identification of highly stable multiple-point mutants. FireProt is composed of stability prediction calculations and back-to-consensus analysis followed by smart filtering of identified mutants. FireProt provides a rapid design of stable proteins while considering the additivity of identified mutations. This enables prediction of multiple-point mutants with significantly enhanced stability and the low introduction of false negatives. FireProt's reliability and applicability were demonstrated by validating its predictions against 1600 mutations from the ProTherm database. Moreover, three proteins belonging to different protein families were selected (human fibroblast growth factor FGF2, haloalkane dehalogenase DhaA and γ-hexachlorocyclohexane dehydrochlorinase LinA) to validate the wider applicability of the FireProt method. Application of FireProt resulted in exceptional stability in all three cases with ΔTm equal to 20°C, 24°C, and 21°C, respectively. FireProt shows wide applicability potentially the proteins with known tertiary structure and homologous sequences. Currently, we work on a completely automatic web server to simplify FireProt usage and to introduce this method to a broad scientific community.

Watching Membrane Protein Folding *in singulo*

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Over the past decade, numerous single-molecule techniques have become available to study biological molecules. In particular, the observation of conformational changes within these biomolecules or during interactions have become directly accessible. Here, I will present how we develop and apply single-molecule FRET (smFRET) as a tool to monitor folding of membrane proteins in a lipidic environment. In particular, we are interested how headgroup chemistry and hydrophobic mismatches affect structure and folding. I will discuss data on the membrane protein Mistic, which folds into detergent micelles on the sub-millisecond timescale and, thus qualifies as an ultrafast folding membrane protein. Furthermore, the folding rates can be greatly influenced by the headgroup chemistry. I will further discuss how smFRET can monitor the structure of a transmembrane helix pair TM3/4 of the cystic fibrosis transmembrane conductance regulator (CFTR) and how the structure is affected by the pathogenic mutation TM3/4 V232D.

The Signaling Mechanism of the Photosensoric Complex of Natronomonas pharaonis Studied by EPR Spectroscopy and Coarse Grained Molecular Dynamics Simulations

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Recent developments including pulse techniques, multi-frequency approaches and new spin labeling techniques make electron paramagnetic resonance (EPR) spectroscopy an attractive approach for studying the structure and conformational dynamics of large biomolecular complexes and biomolecule/nanomaterial hybrid systems. EPR spectroscopy of spin label side chains introduced into a protein or nucleic acid provides structural and dynamic information for restraint modeling of protein domains, protein-protein or protein-nanomaterial interaction sites and their conformational changes with high temporal and spatial resolution. Double electron-electron resonance (DEER) spectroscopy provides distance distributions making this approach very valuable for determining heterogeneity of complex systems, e.g., of membrane protein complexes in their native environment or for characterizing thermodynamic equilibria of protein conformations [1]. Here, we review our studies on the signal transfer mechanism in the halobacterial phototaxis sensory rhodopsin-transducer complex from *Natronomonas pharaonis*, *Np*SRII-*Np*HtrII [2]. Time resolved detection of inter-spin distance changes upon light activation reveals conformational changes of *Np*SRII, and uncovers the mechanism of the signal transfer from *Np*SRII to and along the associated transducer *Np*HtrII [3]. The experimental data together with coarse-grained molecular dynamics simulations of a trimer of receptor/transducer dimers suggest a signaling mechanism based on dynamic allostery [4]. Light triggered inter-spin distance changes in a related system, the light-gated ion channel channelrhodopsin-2, which is one of the most prominent optogenetic tools, reveal a light-induced movement of the transmembrane helix B [5] opening the ion pathway. This provides a new mode of rhodopsin conformational change, which adds to the known displacement of helix F observed in bacteriorhodopsin [6] and *Np*SRII [2].

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GPCR dimerization and activation studied in sequential multiscale simulations

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Homo- and heterodimerization of G protein coupled receptors (GPCRs) is reported in a continuously growing number of biomolecular studies [1]. The chemokine receptor CXCR4 is supposed to form dimers for proper biological activity. Overexpression of CXCR4 has been shown to lead to metastasis and further CXCR4 is known to be a major receptor for HIV-1. CXCR4 signaling was abolished, if the dimerization was modulated by removing cholesterol or by a treatment with TM4 peptides.
We address the dimerization of CXCR4 in pure phospholipid bilayers and in cholesterol-rich membranes [2]. Using hundreds of molecular dynamics simulations [3], we show that CXCR4 dimerizes promiscuously and that the dimerization patterns are dramatically affected by cholesterol. Addition of cholesterol drastically affected the dimerization: cholesterol enabled the formation of a new cholesterol-induced TM3,4/TM3,4 dimer by intercalation at the dimer interface. Activation-competent CXCR4 dimers are suggested to require binding via this TM3,4 interface. Our study elucidates a molecular relationship between the local membrane composition and its influence on GPCR signaling due to modulations of the receptors' dimerization patterns [2].
Receptor activation was studied for the $S1P_1$ receptor. Here, using sequential multiscale simulations [4], we analyzed the binding pathway of S1P to the $S1P_1$ receptor and the coupled receptor activation. It is shown that S1P may access the receptor binding pocket directly from the solvent. Lifting of the bulky N-terminal cap region precedes the initial S1P binding. Glu121 guides S1P penetration and is together with Arg292 responsible for the stabilization of S1P in the binding pocket, in agreement with experimental predictions. The complete binding of S1P is followed by receptor activation: Trp269 moves toward transmembrane helix 7, resulting in the formation of an enhanced hydrogen bond network in the lower part of TM7. Subsequently, the intracellular binding pocket is opened enabling G protein binding. Analysis of the force distribution network in the receptor yields a detailed molecular picture of the signal transmission network upon agonist binding.

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Interactions between interferon-gamma and extracellular portions of its two receptors R1 and R2

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Signaling of interferon-gamma (IFNg), cytokine important in innate and adaptive immune response, depends on forming a ternary complex with two receptors, ubiquitously expressed R1, and tightly regulated R2. I will present our bioinformatic and biophysical studies of interactions between IFNg, and the extracellular portions of its two receptors. Our computer-aided modulation of R1 affinity to IFNg [1, 2] led to seven-fold increase of the interaction affinity (measured as Kd). These computationally designed variations of R1 residues and their experimental testing led to a deeper understanding of specificity of interactions. Later on, we concentrated on so far poorly described R2 receptor and solved its crystal structure (PDB 5eh1 [3]). Ability to express this molecule allowed us for the first time characterize the ternary complex between IFNg and receptors R1 and R2. The IFNg/R1/R2 ternary complex was studied by small angle X-ray scattering (SAXS) and other biophysical techniques; its crystallization has so far been unsuccessful. Binding between IFNg and its receptors measured by microscale thermophoresis (MST) confirmed the high-affinity binding between IFNg and R1 but much weaker one between the binary IFNg/R1 complex and R2. The weaker binding of R2 to IFNg/R1 is supported by the results of our SAXS experiments. Their interpretation provided a few possible models of the ternary complex none of which confirms the expected symmetrical binding between IFNg homodimer with two molecules of R1 and two molecules of R2 with 2:2:2 stoichiometry. Instead, both SAXS and mass spectrometry of fragments after chemical crosslinking point to surprising 2:2:1 stoichiometry and highly asymmetric binding of R2 to the binary IFNg/R1 complex. The binding mode of IFNg/R1/R2 and its SAXS model are being tested by measuring affinities of the ternary complexes with several specifically designed R1 and R2 variants.

Acknowledgements. This study was supported by institutional grant to IBT RVO86652036, and by the Czech Science Foundation project 16-20507S.

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The Grateful Infrared – Novel IR techniques to probe the functional changes of membrane proteins

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The catalytic activity of proteins is a function of structural changes. Very often these are as minute as protonation changes, hydrogen bonding changes and amino acid side chain reorientations. To resolve these, a methodology is afforded that not only provides the molecular sensitivity but allows to trace the sequence of these hierarchical reactions at the same time. I will showcase results from time-resolved IR spectroscopy using quantum cascade lasers which was applied to channelrhodopsin, which represents the first light-activated ion channel to found the basis of the new and exciting field of optogenetics. Finally, I shall provide an outlook towards novel experimental approaches like THz pump / IR probe spectroscopy or near-field IR nanoscopy (see figure below) that are currently developed in my lab. We believe that some of these approaches have the potential to provide new science.

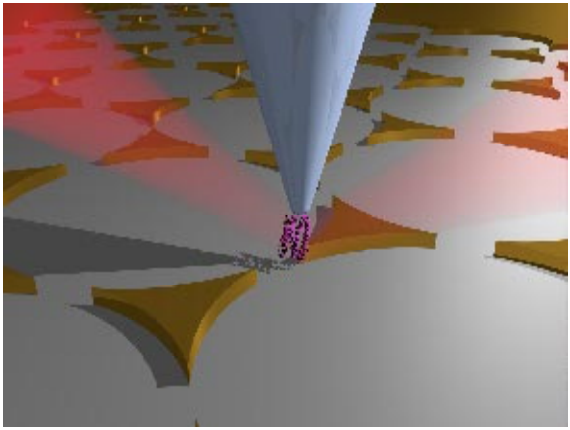

Figure 1: Towards single molecule IR spectroscopy of proteins: Bacteriorhodopsin is tethered to gold nanoantenna serving as a plasmonic surface to enhance the vibrational response. The protein's vibrational spectrum will be probed in the optical near-field by IR radiation scattered at the tip of an atomic force microscope.

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Polymer-Bounded Nanodiscs for Membrane Biophysics

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Once removed from their natural environment, membrane proteins depend on membrane-mimetic systems to retain their native structures and functions. To this end, lipid-bilayer nanodiscs that are bounded by styrene/maleic acid (SMA) copolymers have been introduced as alternatives to liposomes for *in vitro* membrane-protein research [1]. Although these nanodiscs preserve a lipid-bilayer core, they appear to be much more dynamic than other membrane mimics. In this presentation, I will address two aspects related to polymer-based lipid-bilayer nanodiscs.

In the first part, I introduce an alternating diisobutylene/ maleic acid (DIBMA) copolymer that shows equal performance to SMA in solubilizing phospholipids, stabilizes an integral membrane enzyme in functional bilayer nanodiscs, and extracts proteins of various sizes directly from cellular membranes. Unlike aromatic SMA, aliphatic DIBMA has only a mild effect on lipid acyl-chain order and dynamics, does not interfere with optical spectroscopy in the far-UV range, and does not precipitate in the presence of low millimolar concentrations of divalent cations [2].

The second part of the presentation is devoted to the kinetics of lipid exchange among polymer-bounded nanodiscs. By using time-resolved Förster resonance energy transfer and small-angle neutron scattering, we find that nanodiscs exchange lipids not only by monomer diffusion but also by fast collisional transfer. Thus, polymer-based nanodiscs are highly dynamic equilibrium rather than kinetically trapped assemblies, which sets them apart from other membrane mimics and has important implications for studying protein/lipid interactions [3].

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Calcium directly regulates phosphatidylinositol 4,5-bisphosphate headgroup conformation and recognition

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The orchestrated recognition of phosphoinositides and concomitant intracellular release of Ca^{2+} plays a crucial role in every regulatory aspect of cellular life, including membrane homeostasis, vesicle trafficking, as well as secretion. Although the overall effect of Ca^{2+} on phosphoinositide lateral organization has been intensely investigated, the mechanism of these interactions at the molecular-level and its significance in cellular signaling remain unclear. We therefore investigated the direct interaction of Ca^{2+} with phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), the main lipid marker of the plasma membrane. Strikingly, phospholipase C delta 1 pleckstrin homology domain (PLC-PH), $PI(4,5)P_2$-specific lipid sensor, fails to recognize $PI(4,5)P_2$ in the presence of Ca^{2+}, while Mg^{2+} has little, if any effect. Consistently, vibrational sum frequency spectroscopy and atomistic molecular dynamics simulations for the first time reveal in molecular detail how Ca^{2+} binding to the $PI(4,5)P_2$ headgroup and carbonyl regions leads to confined lipid headgroup tilting and conformational re-arrangements. We rationalize these findings by the ability of calcium to block a highly specific interaction between PLC-PH and its lipid receptor $PI(4,5)P_2$, encoded within the conformational properties of the lipid itself.

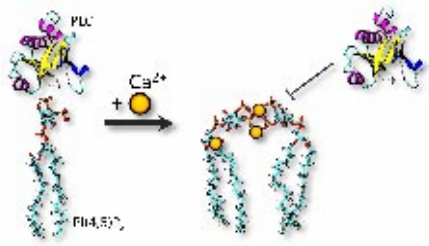

Figure 1: model of calcium effect on $PI(4,5)P_2$ conformation and recognition. In the absence of calcium, $PI(4,5)P_2$ is available for PLC-PH domain binding. When the calcium (yellow circles) is present, it binds to $PI(4,5)P_2$ headgroup phoshates, as well as to the carbonyl groups, promoting clustering of the $PI(4,5)P_2$ molecules. Moreover, calcium causes conformational changes of the $PI(4,5)P_2$ molecule, particularly headgroup tilting. In this calcium-bound state, $PI(4,5)P_2$ is not recognized by PLC-PH domain.

Impact of GM_1 nanodomains in the oligomerisation of membrane bound Aβ monomers

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Oligomers of the β-amyloid (Aβ) peptide are thought to be implicated in Alzheimer's disease (AD). Membranes of neurons may mediate the oligomerisation of Aβ present in brain. Using the single-molecule sensitivity of fluorescence, we address the oligomerisation of Aβ monomers on lipid bilayers containing essential components of the neuronal plasma membrane. We find that sphingomyelin triggers the oligomerisation of Aβ and that physiological levels of GM_1, organized in nanodomains, do not seed oligomerisation. Moreover, GM_1 prevents oligomerization of Aβ counteracting the effect of sphingomyelin. Our results establish a preventive role of GM_1 in the oligomerisation of Aβ suggesting that decreasing levels of GM_1 in the brain, e.g. due to aging, could lead to reduced protection against Aβ's oligomerisation and contribute to AD onset [1].

In addition to the new insights into the molecular mechanism(s) that may be involved in AD, it should be pointed out that this work contains a further important novel finding. We uncovered the existence of nanoscopic heterogeneities (radius 8-26 nm) in microscopically homogenous membranes, unresolvable by super-resolution microscopy. This was achieved by a combination of Monte Carlo Simulations, FLIM-FRET [2] and FCS techniques [3] using our recently developed fluorescent ganglioside analogues [4].

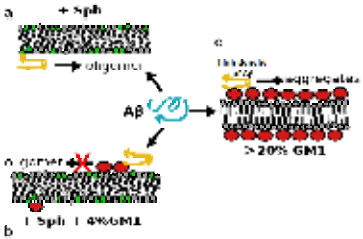

Figure 1: Formation of toxic Aβ oligomers can be differently influenced by different local GM1 organisation. (a) and (b) depict the inhibitory effect of low concentrations of GM1on oligomerisation; (c) depicts the catalysing effect of high concentrations of GM1 on oligomerisation.

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Anomalous Interactions of Amyloids with Surfaces

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Alzheimer's Disease (AD) is the most common form of dementia in the elderly. Currently, 1 in 8 people above the age of 65 suffer from this disorder and the total number of people with AD is expected to grow to 115 million people in 2050 with treatment costing over $2,000 billion/year. However, the molecular understanding of the disease initiation and progress is missing, which complicates the development of treatment. We investigated the behavior of amyloid fibrils and its components, Abeta peptides, in the presence of phospholipid membranes and nanoparticles using molecular simulations supported by fluorescence and crystal microbalance experiments. We demonstrated that sphingomyelin lipids trigger the aggregation of Abeta, while GM_1 glycolipid can prevent it.[1] However, the effect of GM_1 is concentration dependent and high concentrations of GM_1 promote the formation of Abeta fibrils. This is in agreement with the observed non-linear effects of surfaces on both fibril adsorption and kinetics of its formation.[2] In particular, competition between the bulk and surface nucleation and growth results in the retardation of aggregation at weakly attractive surfaces, but aggregation acceleration at strongly attractive surfaces. Moreover, fibrils of a certain length were found to repel, while other lengths attract to the surface due to a competition between electrostatic attraction and excluded volume repulsion. These findings offer molecular insight into complex interaction between Abeta and membranes/surfaces and may be used to control the fibril formation in diverse systems.

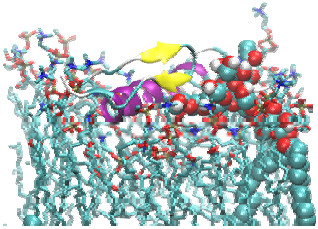

Figure 1: Snapshot of Abeta peptide at the phospholipid membrane with sphingomyelin

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Tear Film Lipid Layer: a molecular-level view

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The ocular surface of the human eye is covered and protected by the tear film, an aqueous multilayered structure [1]. The very outer interface between the tear film and air is coated by the tear film lipid layer (TFLL), a relatively thin lipid-rich region. Its main role is to reduce surface tension of the tear film. Furthermore, it helps with the tear film re-spreading after blinks, provides a smooth optical surface, and prevents water evaporation. By reducing tear film surface tension and assisting in film re-spreading, TFLL is instrumental for maintaining film stability. TFLL deficiencies lead to evaporative dry eye syndrome, one of the commonly reported eye ailments.

Composition and structure of the TFLL are still under debate. This stems from the fact that the tear film as a whole and the TFLL in particular, are complex and dynamic assemblies. They refresh with every blink, undergo complicated time evolution, and eventually rupture if blinking is suppressed. Hence, it is practically complicated to assess macroscopic and microscopic properties of the TLFF in vivo.

We introduced an in silico model of the TFLL that is able to capture key molecular-level characteristics of the tear lipid film [2]. By employing this model in coarse grain MD simulations, we investigated overall structure of TFLL including the role of individual lipid classes on properties and stability of the film [2,3]. Moreover, we examined interaction of TFLL with lysozyme which is the most abundant protein present in tears. Our results suggest that lysozyme may incorporate into TFLL and hence play an important role in enhancing the film stability. Our theoretical studies were accompanied by experimental Langmuir balance measurements coupled with fluorescence microscopy.

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Cell Penetration and Membrane Fusion: Two Sides of the Same Coin

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First, molecular dynamics simulations, together with fluorescence spectroscopy and biomimetic colorimetric assays, have been performed in search of explanations why arginine rich peptides with intermediate lengths of about ten amino acids translocate well through cellular membranes, while analogous lysine rich peptides do not. We observe a strong tendency of adsorbed arginine (but not lysine) containing peptides to aggregate at the bilayer surface. We suggest that this aggregation of oligoarginines leads to partial disruption of the bilayer integrity due to the accumulated large positive charge at its surface which increases membrane-surface interactions due to the increased effective charge of the aggregates. As a result, membrane penetration and translocation of medium length oligoarginines becomes facilitated in comparison to single arginine and very long polyarginines, as well as to lysine containing peptides.

Second, we aim at understanding of interactions of calcium with lipid membranes at the molecular level, which is of great importance in light of the involvement in calcium signaling, association of proteins with cellular membranes, and membrane fusion. Time-resolved fluorescent spectroscopy of lipid vesicles and second harmonic generation spectroscopy of lipid monolayers are used to characterize local binding sites of Ca^{2+} in zwitterionic and anionic model lipid assemblies while dynamic light scattering and zeta potential measurements are employed for macroscopic characterization of lipid vesicles in calcium-containing environment. To gain an atomistic-level information about calcium binding, the experiments are complemented by molecular simulations that utilize an accurate force field for calcium ions with scaled charges effectively including electronic polarization effects. We demonstrate that the membranes have very high calcium-binding capacity, with several types of binding sites present, with important implications for calcium buffering, synaptic plasticity, and protein-membrane association.

Lipid glue in clathrin adaptor assembly and new opportunities at XFELs

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Viruses affect basically all organisms on earth. Some are detrimental to human development, whereas those targeting pathogenic bacteria or crop pathogens can be beneficial for us. Many viruses use clathrin coated vesicles to enter host cells. Clathrin, plasma membrane and cytoskeleton are linked via clathrin adaptor proteins.
Native mass spectrometry (MS) was used to study lipid binding of two clathrin adaptor proteins, which were shown to interact in a lipid dependent fashion. Furthermore, the lipid mediated assembly pathway was investigated with native MS in conjunction with crystallography, DLS, ITC and SAXS. How native MS and X-ray free-electron lasers (XFELs) can play together will be illustrated.
Additional lipid binding sites were identified, which are required for complex assembly. Defined complex stoichiometries are observed and are conserved between species (from yeast to human). Assembly starts with lipid dependent formation of a core structure, to which the second protein attaches. The complex then gets extended reaching its final stoichiometry (8+8+lipid), which is the most stable structure as was confirmed in time-resolved native MS. It turned out that even chimeric species from distinct host organisms can be formed despite low sequence homology hinting at a lipid mediated assembly interface.
Native MS delivers sufficient amount of ions to enable diffraction before destruction or fragmentation for top-down MS at XFELs. Results from feasibility studies and first dissociation tests at a soft XFEL are presented.

Detecting protein structures, ensembles, and dynamics in SAXS/WAXS data: combining MD simulations with Bayesian inference

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Small- and wide-angle X-ray scattering (SWAXS) is in principle capable of detecting protein structures and ensemble in solution, even in a time-resolved manner. However, the interpretation of the signals remains challenging due to (i) low information content of the signals, (ii) scattering contributions from the solvent, (iii) unclear confidence intervals structural models fitted against the data. Such problems lead to a high risk of overfitting and, hence, of drawing unfounded conclusions from the data.

We aim to overcome such problems by combining SWAXS data with explicit-solvent molecular dynamics (MD) simulations and Bayesian inference. Specifically, we have developed methods for SWAXS curve predictions and for structure refinement fully based on explicit-solvent MD [1,2]. The simulations provide an accurate physical model for the hydration layer and excluded solvent, thus avoiding any solvent-related fitting parameters. In addition, MD simulations restrain the biomolecule into conformations of acceptable free energy, thereby strongly reducing the risk of overfitting the data. The methods are illustrated using systems of proteins, protein/detergent complexes, and detergent micelles. In addition, simulations were used to interpret femtosecond time-resolved SWAXS data following CO photodissociation in myoglobin, thus tracking the ultrafast dynamics in solution [3]. Very recently, we combined MD simulations with Bayesian inference, providing a statistically founded interpretation of SAXS data. The method rigorously (i) quantifies the ambiguity of fitted models, (ii) correctly weights the experimental data vs. prior physical knowledge, and (iii) provides route to detect the heterogeneity of a protein ensemble in solution [4].

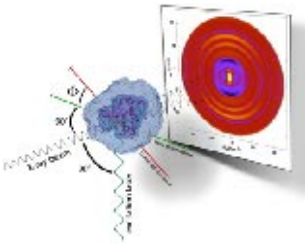

Figure 1: Illustration of calculations of time-resolved WAXS signals after photoexcitation.

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The 14-3-3 protein-dependent regulation of neutral trehalase Nth1

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Trehalases hydrolyze the non-reducing disaccharide trehalose amassed by cells as a universal protectant and storage carbohydrate. Recently, it has been shown that the neutral trehalase Nth1 from yeast *Saccharomyces cerevisiae* is activated in a protein kinase A (PKA)- and calcium-dependent manner in a process that requires binding of Nth1 to the scaffolding protein 14-3-3. To provide a detailed mechanistic insight into a role for 14-3-3 in activating *S. cerevisiae* Nth1, we determined the crystal structure of phosphorylated full-length Nth1 bound to yeast 14-3-3 protein Bmh1. The crystallographic analysis, together with activity and time-resolved fluorescence measurements, indicates that binding of Nth1 by 14-3-3 enables proper positioning of the calcium-binding and the catalytic domains of Nth1. This, in turn, stabilizes the flexible loop that is part of the active site of Nth1 and provides a tyrosine residue important for the catalysis. Our work highlights the ability of the 14-3-3 protein to modulate the tertiary structure of bound protein partner and provides a useful clue for elucidating the molecular mechanism of the 14-3-3-dependent regulation of yeast Nth1.

Proteins in crowded environments

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The pronounced interaction of proteins with other macromolecules can cause significant changes to protein properties such as translational mobility, for example, or their distribution of conformational states. Accordingly, the study of proteins in macromolecular environments that typically very closely resemble physiological conditions or cell-like environments is of great interest. In theory, confocal fluorescence microscopy enables the selective analysis of selected proteins in such environments. In practice, however, a number of methodological difficulties and artifacts of technical measuring need to be accounted for or circumvented, in order to characterize proteins reliably and quantitatively [1]. Besides measurements made directly in living cells, which are technically complex and which typically do not deliver the same kind of data quality as in vitro measurements, cell-like environmental conditions can also be emulated by non-biological macromolecules (e.g. PEG, Ficoll). Using highly concentrated solutions of theses artificial crowder molecules at least two major effects of crowded solutions are incorporated: excluded volume effects and increased solvent viscosity.
Various protein properties observed in solution of different artificial crowder molecules are presented and discussed. In this respect the relative sizes of proteins and crowder molecules play a major role, since the effective viscosity of the crowder solutions is strongly depending on that size relation (Fig. 1).

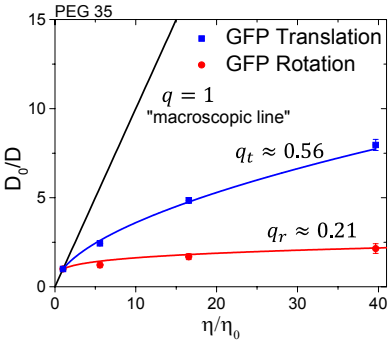

Figure 1: Translational and rotational mobility of green fluorescence protein (GFP) in an artificial crowder solution (PEG 35) measured as a function of crowder concentration. We observe a pronounced deviation from the Stokes-Einstein relation ("macroscopic line") if the crowder (35 kDa) molecules and the diffusing protein (GFP with 27 kDa) exhibit similar sizes.

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Use of fluorescence spectroscopy in synthetic biology

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Enzymes' substrate specificity is strongly connected to the active site surroundings and properties. We report two fluorescence methods that specifically senses hydration and mobility in the active site vicinity of dehalogenase enzymes: A) "Time-dependent fluorescence shifts" (1,2) of environmental sensitive dyes, which are made part of a substrate for the enzymatic reaction, yielding a covalent enzyme-dye complex. B) Decomposition of steady-state spectra of a fluorescent unnatural amino acid (3). Here, the dye is site-specifically incorporated into the protein structure. Both methods are sensitive to the architecture of the tunnel mouth and report substantially different hydration and mobility among different dehalogenase enzyme mutants.
We us this fluorescence approaches to understand why natural enzymes have superior selectivity than enzymes created by De novo protein design. We address this question by transplanting the active site from a dehalogenase enzyme with high enantioselectivity to a nonselective dehalogenase enzyme. Protein crystallography confirms that the active site geometry of the redesigned dehalogenase matches that of the target, but its enantioselectivity remains low. Time-dependent fluorescence shifts and computer simulations revealed that dynamics and hydration at the tunnel mouth differ substantially between the redesigned and target dehalogenase. Our results emphasize the importance of dynamics and hydration for enzymatic catalysis and rational protein design (4).

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THz Spectroscopy and Solvation Science

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The majority of chemical reactions – among those many that are central to important industrial processes – and virtually all biological processes, take place in a liquid-state environment. Solvents– with water being the most prominent – are used to "solvate" molecular species from reagents to proteins and thereby transfer these as "solutes" into the liquid state. Understanding "the role of water in the myriad of processes – from catalysis to molecular recognition "was addressed as one of the main challenges for chemistry in next century. Now, we witness the emergence of Solvation Science as a new interdisciplinary field to understand the influence of solvation on reactions, the function of biomolecules, and processes at liquid-solid interfaces.

Water's flexible network enables it to adapt its structure and dynamics. Hydration water makes significant contributions to the structure and energy of proteins and provides a responsive surrounding, which allows for conformational changes. In particular, water may hold the key to the way proteins interact, fold, bind substrates, and aggregate. Water at protein interfaces (hydration water or interfacial water) has been shown to thermodynamically stabilize the native structure of bio-macromolecules, to affect protein flexibility, and to contribute to molecular recognition in enzyme catalysis. Protein-water interactions are now known to shape the "free energy folding funnel" that drives protein folding.

We could show that THz absorption spectroscopy is a powerful tool to probe hydration dynamics of biomolecules. Under ambient, physiologically relevant conditions 90% of the modes which contribute to the total entropy of the solvated protein are captured by the low frequency modes of the protein/solvent, i.e. the vibrational density of states (VDOS) between 0 and 10 THz (300 cm^{-1}). I will present examples for low frequency spectra of hydration water around solutes and explain how these provide sensitive probes of hydration dynamics. Transient THz spectroscopy can be used to record snapshots of the low frequency spectrum of a solvated protein subsequent to initiation of the protein folding, thus capturing changes during hydrophobic collapse. We propose that water is not just a passive spectator solvent in biological processes, but has a vital function in most biomolecular and cellular processes.

Single-molecule insight into target recognition by CRISPR-Cas systems

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The recently discovered CRISPR-Cas enzymes are considered to be revolutionary tools for diverse biotechnological and medical applications, such as genome editing and transcriptional reprogramming. Central part of these enzyme systems are large effector complexes harboring a short RNA that encodes the binding specificity for a given nucleic acid target. Target binding and recognition involves base pairing of the RNA with the target strand. On double-stranded DNA, this causes local target unwinding. By exchanging the RNA sequence, the target specificity of the effector complex can be easily reprogrammed even on genome-wide scales. The target recognition is, however, highly promiscuous, such that multiple mispairs between RNA and target strand are tolerated. This causes undesired recognition of wrong, so-called off-targets.
Aim of our investigations is to understand the target recognition by CRISPR-Cas effector complexes in a fully quantitative manner to avoid off-targeting. Using single-molecule twisting experiments with near base-pair-resolution, we probe the recognition of sites bearing single mismatches to a given RNA. This allows us to detect the dynamics and the extent of the RNA base-pairing with the target. We can demonstrate that the target recognition is a highly dynamic zipping process between RNA and DNA that always originates from a sequence element upstream of the target. Upon reaching a mismatch site, the zipping stalls and the intermediate often collapses in a length dependent manner. Upon full zipping of the RNA along the target, a conformational change within the effector complex is triggered that causes DNA degradation. Thus, the complex acts as a guard that verifies whether the zipping was eventually successful. Based on these observations we developed a simplistic model to describe the thermodynamic equilibrium and the dynamics of the target recognition process. Combining the modeling with quantitative data about the torque-dependent life-times of zipping intermediates, we obtain fundamental parameters of the target recognition process, such as the underlying zipping rate and the energetic penalties introduced by a single mismatch. This provides the basis to develop quantitative off-target predictors for CRISPR-Cas enzymes and other systems that employ such zipping processes.

No Need to FRET: Observing Membrane Protein Structure and Function by Polarization Fluorescence Microscopy

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Membrane proteins are a diverse group of proteins of high scientific and practical interest, but they are difficult to study because of their requirement for the cell membrane. The technique of two-photon polarization microscopy (2PPM) developed by our laboratory[1] takes advantage of the cell membrane requirement in order to allow sensitive observations of membrane protein function, as well as making insights into membrane protein structure[2, 3].

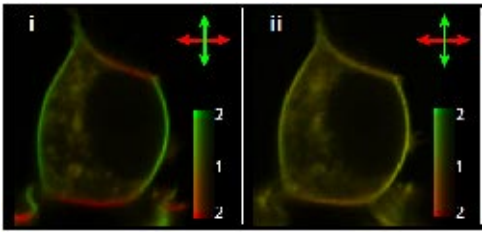

Figure 1: An HEK293 cell expressing a fluorescently labeled G-protein subunit Gαi1, imaged by two-photon polarization microscopy. Red and green colors indicate fluorescence excited by horizontally and vertically polarized light, respectively. Left: before activation of the G-protein signaling cascade; right: after activation by an agonist.

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Novel chemical biology methods to study GPCRs one molecule at a time

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The superfamily of G Protein-Coupled Receptors (GPCRs) is one of the largest in the human genome. GPCRs are targets for about a quarter of all prescription drugs. Recent progress in the structural biology of GPCRs renews the interest in rational drug design and discovery. Rational design of "functionally selective" drugs or ligands presents a particularly difficult problem. Agonist ligands frequently display functional selectivity where activated receptors are biased to either G protein- or arrestin-mediated downstream signaling pathways. However, functional characterization of GPCRs has largely been limited to cell-based high-throughput screening assays. The complexity of the cellular milieu introduces biological variables that make quantitative analysis of structure and dynamics of ligand-receptor complexes that underlie functional selectivity virtually impossible. We are developing single-molecule fluorescence methods to eliminate this biological noise and study such complexes in biochemically-defined systems.[1] Recent methodological advances enabled us to express GPCRs with genetically-encoded unnatural amino acids (uaas). We showed that bioorthogonal functional groups on these site-specifically introduced uaas might be used for bioorthogonal reactions. Among a broad spectrum of bioorthogonal reactions, strain-promoted azide-alkyne cycloaddition (SpAAC) with the uaa *p*-azido-phenylalanine and dibenzocyclooctyne (DIBO) reagents is specific and efficient and presents the best option to obtain stoichiometric receptor-fluorophore conjugates.[2] We designed and built a fully-automatic, multi-color, single-molecule detection fluorescence microscope for long-timescale, multiplexed data acquisition using a multi-well tethered receptor "chip" system. Our convergent technology platform presents a new approach for single-molecule fluorescence studies of the kinetics and dynamics of GPCR signalosomes.

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SELECTED TALK ABSTRACTS / POSTER ABSTRACTS

Ultrafast protein folding through polar interactions in membrane-mimetic environments

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Proteins fold on time scales from hours down to microseconds. Ultrafast folding of proteins requires favorable interactions - intrinsic and extrinsic factors sculpt the folding path Intrinsically size, topology and sequence have been summarized in first principles, while other contributing factors such as the folding environment remains often neglected. This is particularly relevant for membrane-interacting proteins that require the highly complex, anisotropic environment of a lipid membrane or membrane mimetic to fold. Ultrafast folding especially in case of membrane proteins involves further challenges posed by the hydrophilic/hydrophobic interface, heterogeneous lipid moieties, interactions involved at the vicinity of water-headgroup-hydrocarbon-core interfaces. Hence, it becomes evident to segregate interaction contributions posed by polar and non-polar components presented at these interfaces that modulate the folding barrier and stability of proteins. To understand these influences we monitored the kinetics and stability of the membrane-interacting protein Mistic in various detergent micelles and also in absence of a hydrophobic phase in aqueous solution. By employing a single-molecule FRET approach to quantify folding and unfolding rates in detergent micelles from equilibrium measurements, we were able to analyze how contributions from polar and nonpolar interactions to the conformations modulate stability and folding dynamics of Mistic. Our results demonstrate that, although both hydrophobic and polar interactions contribute to the thermodynamic stability of Mistic, they exert opposing effects on the folding free-energy barrier: hydrophobic burial in the aliphatic core stabilizes the folded state but not the transition state, thus slowing down unfolding without affecting folding; by contrast, polar headgroup interactions further stabilize the folded state and, additionally, lower the free-energy barrier to speed up the folding reaction to achieve ultrafast folding on timescales down to 30 μs.

Structural studies of the yeast 14-3-3 protein and neutral trehalase (Nth1) complex

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14-3-3 proteins are family of regulatory molecules which were found in all eukaryotes and are involved in regulation of cell cycle, metabolism, gene transcription or apoptosis. We focused on understanding of the 14-3-3 protein function in the regulation of the neutral trehalase (Nth1, EC 3.2.1.28) from *Saccharomyces cerevisiae*. This enzyme helps yeasts to survive different stress conditions and hydrolyses a storage and protective disaccharide trehalose into two molecules of glucose. The activity of Nth1 is enhanced by the yeast 14-3-3 protein (Bmh1) binding and/or by Ca^{2+} binding to the EF-hand-like motif containing domain of Nth1 [1, 2]. For revealing the mechanism of the 14-3-3- and Ca-dependent activation of Nth1, solving the structure of Nth1 and its complex with 14-3-3 protein we used site-directed mutagenesis, enzyme activity measurements, H/D exchange coupled to MS, SAXS and protein crystallography. We proved that 14-3-3 protein binding induces a rearrangement of the phosphorylated Nth1 molecule and that the separate Ca-binding domain interacts with both the 14-3-3 protein and the catalytic domain of Nth1. The EF-hand-like motif functions as the intermediary through which Bmh1 modulates the function of the catalytic domain of Nth1 [2]. Upon results from crystal structures we identified residues crucial for binding and hydrolysis of trehalose and we tested the importance of so called "lid-loop" (Fig. 1). Our structure of 14-3-3 protein complex with the fully active enzyme Nth1 offers a unique view on Nth1 activation enabling to better understand the role of the 14-3-3 proteins in regulation of other enzymes.

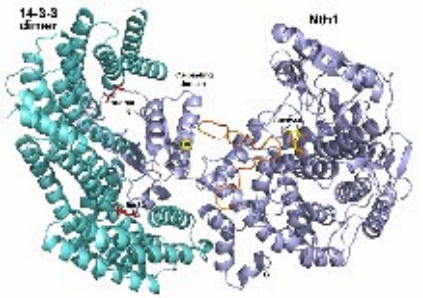

Figure 1: Crystal structure of the pNth1 WT:14-3-3 protein complex. Light blue ribbon diagram of pNth1 WT with "lid-loop" area in orange and phosphorylated pSer60 and pSer83 shown as red sticks. One molecule of sucrose (yellow sticks model) occupies the Nth1 active center and one atom of Ca (yellow sphere) is located in EF-hand-like motif. The ribbon diagram of 14-3-3 dimer is shown in cyan.

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Energetics and mechanism of permeation across the Formate-Nitrite Transporters (FNTs)

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The selective flux of molecules across biological membranes is essential for the normal function of any cell. Membrane transport proteins are typically classified into two big groups: channels and transporters, based on several criteria, such as turnover rates, or the extent of conformational change on permeation. However, the continuous uncovering of the broad spectrum inhabited by such proteins has shown that the boundary between these two groups is not always clear.

We study the formate-nitrite transporters (FNTs), a striking example of a protein family that exists on the border between channels and transporters. Even though they are structurally related to aquaporins, their role is not water permeation, but rather anion permeation, including formate, nitrite, lactate, bicarbonate, and acetate. Furthermore, FNTs are widely expressed in microorganisms, including many pathogens, making them an attractive drug target.

Combining molecular dynamics with quantum chemical and p*Ka* calculations, we show how a tightly controlled protonation event may (i) constitute the selectivity for weak acids, (ii) allow for symport and channel modes of permeation, and thereby (iii) avoid proton leakage via a Grotthus mechanism. This function is enabled without the need for a major conformational transition, but simply by modifying the free-energy landscape of the substrate using the involved proton.

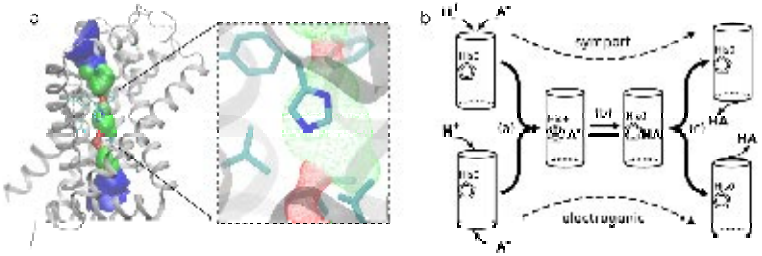

Figure 1: (a) NirC protomer (PDB ID: 4FC4) with HOLE representation of the permeating pore. Enlarged representation of the region around a highly conserved central histidine residue is shown on the right. (b) Proposed mechanism of permeation across the FNTs, which allows for electrogenic channel-like permeation and proton symport.

Trans-membrane pore formation: free energies and reaction coordinates

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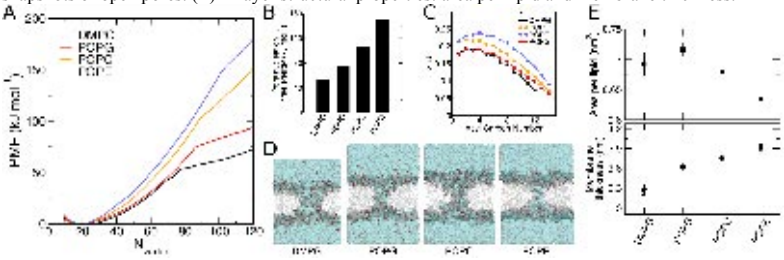
Trans-membrane pores play an important role in various biophysical processes such as membrane permeation, membrane fusion, and antimicrobial peptide activity. In order to quantitatively describe the role of membrane composition and specific phospholipids, we calculate free energies of trans-membrane pore formation via molecular dynamics (MD) simulations for pure and binary lipid mixtures [3]. We find that charged lipids in bilayers favor pore nucleation (fig.1). We show that phospholipid head-groups determine the balance of entropy and enthalpy in the total free energy of pore formation. We resolve the role of phospho-ethanolamine (PE) lipids in pore formation by discussing the energetics and preferred spatial distribution of PE in binary lipids mixtures [3].
Finally, we address the question about the choice of a good reaction coordinate (RC) for reliable and efficient free-energy calculations of pore formation using MD simulations [1,2]. We have previously highlighted the limitations of the available RCs used in free energy calculations [1]. In our recent work, we propose a new RC that samples the opening and closing of a trans-membrane pore without hysteresis, and leads to converged free energies within reasonable simulation timescales [2].

Figure 1: Pore formation for membranes with different phospholipids: relating energetics and structural properties. (A) Potential of mean force (PMF) for pore opening. (B) Pore nucleation free energies. (C) Deuterium order parameter, $|S_{cd}|$ for the saturated palmitoyl chains of the 4 lipids. (D) Final simulation snapshots of open pores. (E) Bilayer structural properties: area per lipid and membrane thickness.



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Calcium directly regulates phosphatidylinositol 4,5-bisphosphate headgroup conformation and recognition

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The orchestrated recognition of phosphoinositides and concomitant intracellular release of Ca^{2+} plays a crucial role in every regulatory aspect of cellular life, including membrane homeostasis, vesicle trafficking, as well as secretion. Although the overall effect of Ca^{2+} on phosphoinositide lateral organization has been intensely investigated, the mechanism of these interactions at the molecular-level and its significance in cellular signaling remain unclear. We therefore investigated the direct interaction of Ca^{2+} with phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), the main lipid marker of the plasma membrane. Strikingly, phospholipase C delta 1 pleckstrin homology domain (PLC-PH), $PI(4,5)P_2$-specific lipid sensor, fails to recognize $PI(4,5)P_2$ in the presence of Ca^{2+}, while Mg^{2+} has little, if any effect. Consistently, vibrational sum frequency spectroscopy and atomistic molecular dynamics simulations for the first time reveal in molecular detail how Ca^{2+} binding to the $PI(4,5)P_2$ headgroup and carbonyl regions leads to confined lipid headgroup tilting and conformational re-arrangements. We rationalize these findings by the ability of calcium to block a highly specific interaction between PLC-PH and its lipid receptor $PI(4,5)P_2$, encoded within the conformational properties of the lipid itself.

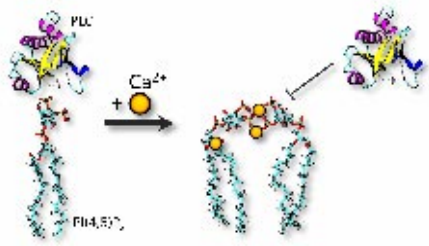

Figure 1: model of calcium effect on $PI(4,5)P_2$ conformation and recognition. In the absence of calcium, $PI(4,5)P_2$ is available for PLC-PH domain binding. When the calcium (yellow circles) is present, it binds to $PI(4,5)P_2$ headgroup phoshates, as well as to the carbonyl groups, promoting clustering of the $PI(4,5)P_2$ molecules. Moreover, calcium causes conformational changes of the $PI(4,5)P_2$ molecule, particularly headgroup tilting. In this calcium-bound state, $PI(4,5)P_2$ is not recognized by PLC-PH domain.

Annotation of DNA Structures by a Newly Formulated DNA Structural Alphabet

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DNA is a structurally flexible molecule and its function depends on the ability to adapt to its binding partners. DNA structural variability goes way beyond the traditionally used classification schema into A-form, B-form, and Z-form. Therefore, we analyzed almost 60 000 dinucleotides in non-redundant crystal structures of "naked" DNA and DNA complexed with proteins available in Protein Data Bank (PDB) to capture the full spectrum of DNA variability The analysis lead to characterization of 44 distinct conformational classes we call NtC (Nucleotide Conformers). We further combined these classes according to their structural similarities into 12 "letters" of a new structural alphabet, CANA (Conformational Alphabet of Nucleic Acids). The alphabet simplifies the human understanding of DNA structures and streamlines its conformational analysis. Dinucleotides are classified into NtC and CANA classes by an automated assignment protocol available at websites dnatco.org [1] and dolbico.org. The agreement between the known conformation classes and conformation of an analyzed structure is quantified by a newly defined validation score called confal. The dinucleotide conformers enabled us to describe the structural variability of typical DNA structures such as Dickerson-Drew dodecamer, structural models based on the fiber diffraction, and guanine quadruplexes. Further, we found that periodic occurrence of one of the defined conformers can explain wrapping of DNA around the histone core.

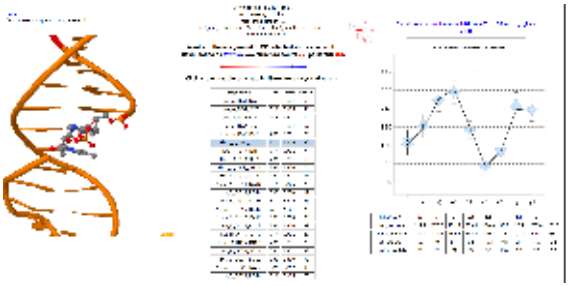

Figure 1: The structure of the Dickerson-Drew dodecamer, PDB code 1bna [2], as can be seen at dnatco.org after assignment into NtC and CANA, with calculated values of confal.

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Analysis of self-assembly of S-layer protein slp-B53 from Lysinibacillus sphaericus

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The formation of stable and functional surface layers (S-layers) via self-assembly of surface-layer proteins on the cell surface is a dynamic and complex process. S-layers facilitate a number of important biological functions, e.g., providing protection and mediating selective exchange of molecules and thereby functioning as molecular sieves. Furthermore, S-layers selectively bind several metal ions including uranium, palladium, gold, and europium, some of them with high affinity. Most current research on surface layers focuses on investigating crystalline arrays of protein subunits in Archaea and bacteria. In this work, several complementary analytical techniques and methods have been applied to examine structure–function relationships and dynamics for assembly of S-layer protein slp-B53 from Lysinibacillus sphaericus: (1) The secondary structure of the S-layer protein was analyzed by circular dichroism spectroscopy; (2) Small-angle X-ray scattering was applied to gain insights into the three-dimensional structure in solution; (3) The interaction with bivalent cations was followed by differential scanning calorimetry; (4) The dynamics and time-dependent assembly of S-layers were followed by applying dynamic light scattering; (5) The two-dimensional structure of the paracrystalline S-layer lattice was examined by atomic force microscopy. The data obtained provide essential structural insights into the mechanism of S-layer self-assembly, particularly with respect to binding of bivalent cations, i.e., Mg2+ and Ca2+. Furthermore, the results obtained highlight potential applications of S-layers in the fields of micromaterials and nanobiotechnology by providing engineered or individual symmetric thin protein layers, e.g., for protective, antimicrobial, or otherwise functionalized surfaces

References

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Potassium uptake systems in *Candida* species

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Several tens of *Candida* species are opportunistic human pathogens able to cause life-threatening system infection in immunocompromised patients while *C. albicans* possesses among *Candida* species the highest incidence.
Candida species as other living cells maintain relatively high intracellular concentration (200 – 300 mM) of potassium cations, which play an important role in many biological processes such as the regulation of cell volume, pH and membrane potential.
Yeast cells use potassium importers (uniporters, symporters and ATPases) to provide cell with sufficient amount of K^+. We identified the genes encoding putative K^+-importers in genomes of nine *Candida* species. These differ in the number and type of genes for K^+ importers and in their ability to grow on limiting concentration of KCl. But a higher number of genes for K^+-importers does not correlate with better growth upon K^+ limitation.
C. albicans is the only one species whose genome contains genes for all three known types of yeast K^+-importers. Upon heterologous expression in the *S. cerevisiae Δtrk1Δtrk2* strain lacking its own potassium transporters, all of them able were to provide sufficient amount of K^+ to support cell growth and the division.
In two *C. albicans* lab strains (SC5314 and WO-1) *ACU1* gene encoding K^+ -importing ATPase is divided in two ORFs. We found that the mutation resulting in STOP codon breaking this gene is present only in approx. one third of 34 *C. albicans* strains with known genome sequence.
We plan to analyze the variability of genes for all K^+-importers among *C. albicans* strains and follow in their functional characterization to clarify the role in potassium homeostasis of single K^+-importers, which could be promising targets of new antifungals.

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

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The active transport of ions across biological membranes requires their hydration shell to interact with the interior of membrane proteins. However, the influence of the external lipid phase on internal dielectric dynamics is hard to access by experiment. Using the octahelical transmembrane architecture of the copper-transporting P_{1B}-type ATPase from *Legionella pneumophila* (LpCopA) as a model structure, we have established the site-specific labeling of internal cysteines with a polarity-sensitive fluorophore. This enabled dipolar relaxation studies in a solubilized form of the protein and in its lipid-embedded state in nanodiscs. Time-dependent fluorescence shifts revealed the site-specific hydration and dipole mobility around the conserved ion-binding motif. The spatial distribution of both features is shaped significantly and independently of each other by membrane lateral pressure.

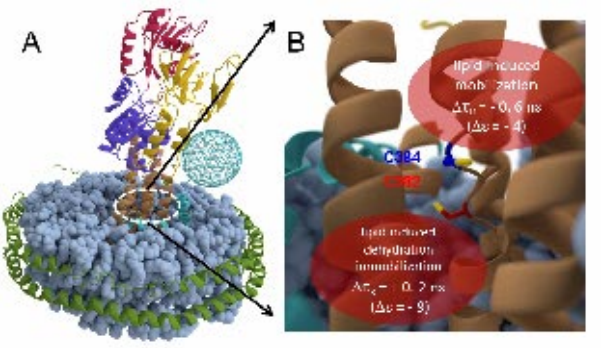

Figure 1: A) schematic of LpCopA reconstituted in a nanodisc. B) close up of the ion-binding site with local assignment of lipid-induced physical changes.

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Membrane interaction and conformational changes of α-synuclein

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α-synuclein (αS) has been identified as the major component of intracellular protein deposits in several neurodegenerative diseases. There is substantial evidence that αS interaction with lipid membranes is pivotal for αS aggregation [1]. We utilize ATR-FTIR difference spectroscopy and solid supported lipid bilayers (SSLB) to study conformational changes of αS wildtype and the splicing variant αS Δexon3 upon membrane interaction. The variant αS Δexon3 lacks the amino acids 41-54. The splicing is suggested to interrupt the protein–membrane interaction [2]. Various fractions of the secondary structure were resolved by amide I band deconvolution. For a short term only, both proteins remain intrinsically disordered in solution as reported before [3]. After several hours, both αS variants adopt heterogeneous structure with a predominant fraction of β-sheets. In contrast to solution, an increased fraction of α-helical structure is observed upon membrane binding for the wildtype protein as well as for the splicing variant. Moreover, β-structures are formed already within several minutes of membrane interaction and grow rapidly, for both variants. Interestingly, the β-sheets of the splicing variant grow much faster than for the wildtype protein. Hence, our findings contradict the assumption that the shorter αS Δexon3 has a reduced membrane interaction compared to the wildtype protein. High sensitivity of ATR-FTIR difference spectroscopy allows simultaneous detection of even slightest changes in the membrane and the protein. We observe profound changes in the absorbance signal of the lipid bilayer groups simultaneously to αS aggregation and membrane interaction. Furthermore, difference spectra indicate that long membrane interaction with αS Δexon3 leads also to negative H_2O difference bands. This is explained by the protein sedimentation on SSLB and the subsequent displacement of water farther from the ATR crystal. However, interaction of αS wt with SSLB leads to positive H_2O difference bands, which indicates water transfer towards the surface of the ATR crystal due to membrane disruption by αS wt. The protein variant αS Δexon3 aggregates faster on SSLB; but there is no indication for membrane disruption. However, the slower aggregating αS wt disrupts the SSLB to some extent.

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Adsorption and aggregation of hIAPP at different self-assembled monolayers

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Misfolding and aggregation of the peptide hormone human islet amyloid polypeptide (hIAPP) plays an important role in the development of type2 diabetes mellitus (T2DM). Different amyloid aggregates can be formed, in particular various fibrillar species and oligomeric particles, all of which are characterized by the intermolecular β-sheets. These aggregates have been found cytotoxic as they may permeabilize and rupture cell membranes [1]. Understanding the interaction of hIAPP with membranes and especially the influence of the membrane properties therefore represents an important goal. Consequently, different model surfaces have been utilized in the past to study the surface-catalyzed aggregation of hIAPP in dependence of the surfaces' physicochemical properties in detail [2].
In this work, we investigate the adsorption and aggregation of hIAPP at self-assembled monolayers (SAMs) on gold surfaces that present different functional groups, i.e., NH_2, OH, COOH, and CH_3. The dynamics of hIAPP adsorption on these surfaces is studied in situ using quartz crystal microbalance with dissipation (QCM-D), while the morphology of the aggregates is analyzed by ex-situ atomic force microscopy (AFM). We observe a strong influence of the functional group on hIAPP adsorption and fibril formation. In particular, the negatively charged COO^- terminated SAM is found to promote fibrillation, in agreement with previous observations on other negatively charges surfaces and membranes [2,3]. In order to obtain a deeper understanding of the relevant mechanisms that control the surface-catalyzed aggregation of hIAPP, these results are compared to additional experiments with rat IAPP that is not as prone to aggregation as the human variant.

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Conservation of folding and association within a family of spidroin N-terminal domains

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Web spiders synthesize silk through a controlled phase and structural transition of soluble fibroins, so-called spidroins, within a spinning gland [1]. Spidroin N- and C-terminal domains (NTD and CTD) are highly conserved across species and glands underscoring their important function in the process of silk synthesis. Yet, sizes, geometries, and mechanical properties of webs built by different spider species vary strongly.

Here, we report the comparative study of folding and association of isolated major ampullate spidroin 1 (MaSp1) NTDs from the black widow spider (*Latrodectus hesperus*), the brown widow (*Latrodectus geometricus*), the golden orb spider (*Nephila clavipes*), and the nursery web spider (*Euprostenops australis*) using steady-state fluorescence, circular dichroism, and stopped-flow fluorescence spectroscopy.

We found that all four domains folded on a sub-millisecond time scale via a conventional two-state mechanism, with significantly reduced stability of the homologue of *N. clavipes*. The rate constant of pH-triggered self-association of the domains and their kinetics of dissociation was conserved. Results showed that, despite substantial differences in the evolution of web geometries and fiber strengths, the process of self-assembly of NTDs originating from different species has conserved energetics and kinetics [2].

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Single-Molecule Studies on CFP-YFP-based biosensors

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Genetically encoded FRET-based biosensors consist of two fluorescent proteins (donor and acceptor) and a sensing domain. If a biological stimulus acts on the sensing domain this is converted into a change in energy transfer [1].
The readout of FRET-based biosensors usually utilizes the ratio of fluorescence emission intensities of the donor and the acceptor upon donor excitation. However, ensemble FRET measurements are biased by varying fractions of donor-only molecules.
We carried out single-molecule measurements on a confocal microscope of a set of CFP-YFP biosensors for measuring the glucose concentration [2] and macromolecular crowding [3]. The glucose binding protein MglB undergoes a conformational change upon glucose binding which alters the energy transfer between the attached CFP and YFP and acts by this as a glucose sensor. In contrast, the crowding sensors have no unique tertiary structure. These sensors are rather forced in a compact state by excluded volume effects.
Single-molecule measurements reveal the distribution of FRET efficiencies of the sensors. Thus, it is possible to observe if all sensor molecules contribute similarly to the signal or if subpopulations are visible. By direct acceptor excitation we sort out donor-only molecules and get unbiased FRET efficiencies. Additionally, we developed an improved two-color coincidence algorithm to quantify the fractions of donor-only, acceptor-only, and donor-acceptor molecules. This coincidence analysis is also applicable to many further fluorescence assays.
In combination with time-resolved anisotropy measurements we aim to understand the sensing mechanisms and, thus, enable a rational sensor design.

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Functional Dynamics in Superoxide Reductase

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Superoxide, $O_2^{\bullet-}$, is a reactive oxygen species (ROS) that can damage biological transition metal compounds of medical or industrial relevance. Superoxide reductases (SOR) catalyze the reduction of $O_2^{\bullet-}$, and, thus, these non-heme iron enzymes represent valuable model systems for the reductive detoxification of ROS.

Here, we applied vibrational spectroscopy and several theoretical methods to provide insights into the structural plasticity and functional dynamics of SOR.[1,2,3] In this respect, structural changes during reductive activation were explored using the example of a homotetrameric 1Fe-SOR.[1,2] Based on infrared difference spectroscopy and density functional theory, amino acid rearrangements close to the active site were revealed, proving the reversible reductive dissociation of an iron-bound glutamate. Moreover, concomitant changes of the protein backbone suggest a concerted structural reorganization. This finding indicates cooperative enzyme activation, and, based on normal mode analysis (NMA), low frequency vibrations are proposed to provide a reaction coordinate for the underlying structural transition.

Using different theoretical methods, we also studied structural dynamics of a prototypical 2Fe-SOR and its influence on electron transfer toward the active site.[3] Based on NMA and essential dynamics analyses, we could show that enzymes of this type are capable of electrostatically triggered domain movements, which may allow conformational proofreading for cellular redox partners involved in intermolecular electron transfer. Moreover, these global modes of motion were found to enable access to molecular configurations with decreased tunneling distances between the active site and the enzyme's second iron center. Using the PATHWAYS model and molecular dynamics simulations, however, we found that electron transfer between the two metal sites is not accelerated under these conditions. This unexpected finding suggests that the unperturbed enzymatic structure is optimized for intramolecular electron transfer, which provides an indirect indication for the biological relevance of such a mechanism. Consistently, efficient electron transfer was found to depend on a distinct route, which is accessible *via* the equilibrium geometry and characterized by a *quasi* conserved tyrosine that could enable multistep tunneling.

In a wider sense, the presented studies demonstrate the functional relevance of structural dynamics, and strategies for analyzing these aspects are provided.[1,2,3]

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On the stability and degradation of DNA origami nanostructures in urea and guanidinium chloride

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DNA origami [1] is an emerging tool with great potential for applications in nanotechnology, biophysics, and structural biology. For DNA origami assembly, a long, single-stranded DNA scaffold is folded into a desired 2D or 3D nanoscale object using base pair complementarity and segmental crossovers of a large number of short oligonucleotides, so-called staple strands. The resulting DNA origami nanostructures may serve as spatially addressable molecular breadboards that enable the controlled arrangement of functional entities with nanometer precision. DNA origami nanostructures thus represent powerful platforms for single-molecule investigations of biomolecular processes [2]. The required structural integrity of the DNA origami may, however, pose significant limitations regarding their applicability, for instance in protein folding studies that require strongly denaturing conditions. Here, we therefore report a detailed study on the stability and degradation of 2D DNA origami triangles in the presence of the strong chaotropic denaturing agents urea and guanidinium chloride (GdmCl) [3].

At room temperature, the DNA origami triangles are stable up to at least 24 h in both denaturants at concentrations as high as 6 M. At elevated temperatures, however, structural stability is governed by variations in the melting temperature of the individual staple strands. The global melting temperature of the DNA origami does thus not represent an accurate measure of their structural stability. Although GdmCl has a stronger effect on the global melting temperature, its attack results in less structural damage than observed for urea under equivalent conditions, which may hint at different mechanisms of action. Therefore, in order to further elucidate the molecular mechanisms of DNA origami denaturation by urea and GdmCl, the effect of Na^+ and Mg^{2+} ions on DNA origami stability in both denaturants was also investigated. While both ions enhance the stability of the DNA origami nanostructures at high concentrations in urea, they are found to promote DNA origami degradation under similar conditions in GdmCl.

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Ca^{2+}–induced Structure Formation in a Disordered Protein

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The MIIA (metal ion-induced autocleavage)-domain of the protein Vic_001052 from the pathogen *Vibrio coralliilyticus*, comprises 173 amino acids and exhibits Ca-dependent auto-proteolytic activity[1]. It shows homology to nodulation proteins which are secreted by Rhizobiacea into plant host cells where they exert Ca-dependent functions. We have studied the structural and energetic aspects of metal protein interactions of the MIIA domain which appear attractive for engineering metal-binding synthetic peptides. Using a non-cleavable MIIA domain construct, we detected very similar structural changes upon binding to Ca^{2+} and Eu^{3+}. We studied the stoichiometry of the Ca^{2+}-Protein binding interaction by Mass Spectroscopy and the thermal denaturation of the Ca-bound state by CD spectroscopy. The metal-bound folded state unfolds reversibly into an unstructured metal-free state similar to the metal-free state at room temperature.

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Determinants of sodium and calcium adsorption onto neutral lipid bilayers and how the cell can use them to modulate hyaluronan–membrane interaction

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Metal cations adsorption to cellular membranes change a number of key functions, such as interaction with charged moieties, cell volume, membrane fusion or cell membrane potential. However, it is unclear how or whether cells regulate this adsorption and hence the related functions through adjusting the local lipid composition of their membranes.We have

We have employed both fluorescence techniques and computer simulations to study how the presence of cholesterol -- a key molecule in inducing membrane heterogeneity -- and temperature can affect the adsorption of sodium and calcium on neutral phosphatidylcholine (PC) bilayers. We find that whereas transient sodium binding is dependent on the sole number of exposed PC head groups, the strong adsorption of calcium is determined by the available surface area of the membrane. Notably, cholesterol plays an indirect role in enlarging the total membrane area, therefore, increasing calcium adsorption, while having no effect on the adsorption of sodium.

These findings improve our understanding of how lateral lipid heterogeneity, that regulates surface charge density, regulates numerous ion-induced processes including adsorption of peripheral molecules such as the usually charged components of the glycocalyx.

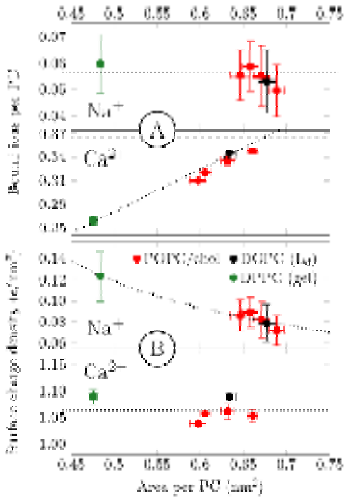

Fig 1: Dependence of cation adsorption on the area per phospholipid.

Glycosaminoglycans (GAGs) are the most abundant sugars of the glycocalyx. GAGs in the glycocalyx can be either free, e.g. hyaluronan, or covalently bound to several transmembrane and extracellular proteins, e.g. heparan sulfate. In all cases, these GAGS are long negatively charged sugar polymers and are often located close to the plasma membrane surface. Therefore, they could potentially interact with the membrane, playing a significant role in its modulation [1]. Very little is known about the nature of this interaction and its biological relevance. Here we focus on unraveling hyaluronan–model membrane interactions, to start to understand their interplay in real plasma membranes.

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Time-domain THz spectroscopy reveals coupled protein-hydration dielectric response in solutions of native and fibrils of human lysozyme

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Structural changes of proteins are crucial for biological function. Among these dynamical structural variations, there is increasing interest in amyloid fibril formation processes that are related to a variety of neurodegenerative diseases in mammals. Here we reveal details of the interaction between human lysozyme proteins, both native and as fibrils, and their water environment by intense terahertz time domain spectroscopy. Using this sensitive tool, we are able to clearly discern between water solutions containing native human lysozyme and fibrils formed from lysozyme. Moreover, with the aid of a rigorous dielectric model we determine the amplitude and phase of the oscillating dipole induced by the THz field in the volume containing the protein and its hydration water. At low concentrations, the amplitude of this induced dipolar response decreases with increasing concentration. Beyond a certain threshold, marking the onset of the interactions between the extended hydration shells, the amplitude remains fixed but the phase of the induced dipolar response, which is initially in phase with the applied THz field, begins to change. These results identify a dynamic hydration shell that might extends up to 42 Å from the surface of the native proteins at low concentrations. The changes observed in the THz response reveal protein-protein interactions mediated by extended hydration layers, which may control fibril formation and may have an important role in chemical recognition phenomena.

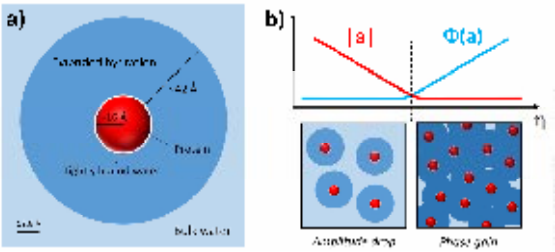

Figure 1: a) Cartoon of a human lysozyme protein (red sphere) in water together with the populations of: i) water molecules tightly-bound to the protein surface (white), ii) extended hydration layers (blue), and iii) unperturbed bulk water (light blue). b) Sketch of the evolution of the modulus and the phase of the induced dipole in a unit volume of solution versus protein concentration. The effect of phase gain at larger concentrations is pictorially represented by darker colors on the bottom right panel.

Solvent effects of H_2O and D_2O on the site-directed fluorescently-labeled P_{IB}-type ATPase CopA from *Legionella pneumophila*

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A fraction up to 20 – 30 % of proteomes is constituted by membrane proteins [1]. This huge amount in combination with their high impact in pathologies makes them a highly important research target. *Legionella pneumophila* CopA (*Lp*CopA) is a member of the P_{IB}-type ATPases and maintains the copper homeostasis in the cell. The elucidation of the crystal structure of CopA in 2011 resolved many unique features of the pump. It consists of four cytoplasmic domains: the nucleotide-binding domain, the phosphorylation domain, the actuator domain and the heavy metal binding domain, together with eight transmembrane domains. The transport cycle of copper across the membrane is briefly characterized by two conformational states E1 and E2. During the process copper is coordinated by cysteines, namely by two ion-binding sites which include C382 and C384 in the CPC motif on M4 [2]. Previously, the functional role of hydration sites in the transmembrane domain of *Lp*CopA was studied and a model was proposed that cysteine 384 facilitates the copper entry and its hydration sites are largely unaffected by the lateral pressure exerted by the membrane. In contrast, cysteine 382 is surrounded by a high fraction of mobile water which is decreased with an increase in lateral pressure. Therefore, this site could act as a flexible switch supporting the copper exit into the lumen [3].
Here, the properties of hydration sites surrounding the membrane buried cysteine 382 in *Lp*CopA were analyzed. To this end, a mutant of the wild-type *Lp*CopA, lacking all cysteine residues except C382, was purified and labeled with the polarity-sensitive fluorescent probe BADAN. Steady-state fluorescence spectroscopy was performed with the labeled protein in micelles solved in either H_2O or D_2O buffer. The isotope exchange was performed by dialysis and verified by Fourier-transform infrared spectroscopy. Comparison of the two isotopes can elucidate hydration water properties of the intra-protein hydration sites. Observation and quantitative analysis of the steady-state fluorescence spectra revealed a small isotope effect.

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Mechanism of attenuation of uranyl toxicity by glutathione in *Lactococcus lactis*

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Both prokaryotic and eukaryotic organisms possess mechanisms for the detoxification of heavy metals, which are found among distantly related species. We have investigated the role of intracellular glutathione (GSH), which in a large number of taxa plays a role in the protection against the toxicity of common heavy metals. Anaerobically grown Lactococcus lactis containing an inducible GSH synthesis pathway was used as a model organism This physiological trait allows study of putative GSH-dependent uranyl detoxification mechanisms without interference from additional reactive oxygen species. By microcalorimetric measurements of the metabolic heat during cultivation, it was shown that intracellular GSH attenuates the toxicity of uranium at a concentration in the range of 10-150 µM; in this concentration range, no effect was observed with copper which was used as a reference for redox-metal toxicity. At higher copper concentrations, GSH aggrevates metal toxicity. Isothermal titration calorimetry reveals the endothermic binding of U(VI) to the carboxyl group(s) of GSH, rather than to the reducing thiol group involved in copper interactions. The data indicate that the primary detoxifying mechanism is the intracellular sequestration of carboxyl-coordinated U(VI) into an insoluble complex with GSH[1]. The opposite effects of GSH on uranyl and copper toxicity can be related to the difference in coordination chemistry of the respective metal-GSH complexes, which cause distinct growth phase-specific effects on enzyme metal interactions.

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From Atom to Cell: Integrating Experimental Results and User Expertise into Computational Modeling

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The three-dimensional structures of proteins evolved to support their dynamical function. Molecular dynamics simulations are well suited to connect function and dynamics to structural data from diverse experimental sources to investigate critical cellular processes occurring on the sub-Å level up to the macromolecular level [1,2].
Sub-Å resolution of catalytic centers of X-ray structures is reached by integration of experimental and theoretical IR spectroscopy. This high resolution enabled an improved understanding of enzyme catalysis demonstrated in the case of the small GTPase Ras, a crucial switch regulating cellular signal transduction [3,4,5].
On the macromolecular level, such high resolution is not as crucial, however structure analysis must deal with highly flexible or multi-conformational domains, which are inaccessible to experiments alone. Therefore, structural data from X-ray crystallography and cryo-electron microscopy are combined with computational modeling techniques. This approach enabled us to obtain nucleotide-bound atomic structure (3.9 Å -7.7 Å resolution) of four different states of the functional cycle of 26S proteasome [6,7], a 2.5MDa multi-subunit molecular machine, which is a key player for protein degradation in cells and an important drug target for multiple diseases.

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Linking Viscosity and TD THz Spectra in Polyether Solvation

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PEOs (Polyethylene oxides, Figure 1 (b) inset) are one of the most versatile polymers and widely used in chemistry, biology and related fields [1]. The specific PEO-water interactions are thought to be the key in understanding, for example, the observed improvement in drug delivery [2]. Our approach towards revealing the origin of the PEO-water interaction is Terahertz (THz) spectroscopic sampling of the aqueous polyether binary mixtures.

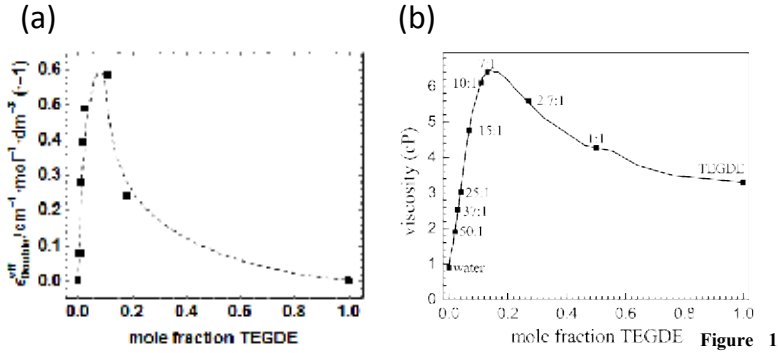

Figure 1 (a) Evolution of the averaged effective molar extinction (0.1–2.3 THz) of water:TEGDE mixtures at 20 °C as a function of mole fraction of TEGDE. The dashed black line is a guide to the eye. (b) Viscosity data for mixtures of water and TEGDE at 25 °C. The labels indicate the molar ratio of water:TEGDE. Adapted from [3].

We have carried out concentration dependent THz absorption measurements of PEO-water (tetraethylene glycole dimethylether, TEGDE) mixtures and found a correlation between changes in the THz absorption (Figure 1 (a)) and the well-known viscosity data (Figure 1 (b)) [3]. The polyether hydration status is linked to its viscosity, which is then directly reflected in the absorption changes in the low-frequency range. At the peak, where x_{TEGDE}=0.1, (corresponding to ~8 waters per TEGDE) all ethers are probably H-bonded, which could give rise to a less flexible water network.

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Membrane integrity and orientation of lipids in solid supported lipid bilayers

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Lipid bilayers play a crucial role in many biological processes. Therefore it is important to know about their properties. To determine these properties we use solid supported lipid bilayers (SSLB) as biomimetic membranes. These are formed by lipid vesicle spreading on a silicon crystal. Using polarized ATR-FTIR spectroscopy we are able to follow the formation and disruption of the SSLB and to determine the orientation of the lipids. The effect of alpha synuclein (αS) aggregation on the membrane properties is of special interest since the disruption of membranes is suggested to be the deleterious mechanism in Parkinson´s disease. By using infrared spectroscopy we are able to determine the changes in the lipid bilayer and the protein simultaneously. Determination of the orientation of secondary structure elements of αS will give further insights into the membrane-protein interaction. The effect of detergents like SDS and the influence of temperature are also under investigation.

The contact bubble bilayer technique for investigation of lipid-protein interactions

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The question on how lipid membranes modulate ion channel function has gained increasing interest in the past years. A good model system to study these protein-lipid interactions is provided by small viral K+ channels. Because of their small size of only 82 and 79 aa per monomer the two channels Kcv_{NTS} (K^+ channel *chlorella* virus) and $Kmpv_{12T}$ (K^+ channel *micromonas pussilla* virus) are fully embedded in the lipid bilayer [1], which maximizes the interplay between protein and lipid.
With the contact bubble bilayer method [2], a modification of the droplet interface bilayer technique [3], very small solvent free bilayers (~50 µm in diameter) can be easily created. Single channel proteins were routinely reconstituted in these bilayers and functionally characterized.
With this technique it is possible to create non-symmetrical bilayers with two different phospholipids on cis and trans. The technique also allows a stable formation of bilayers from lipids like DPPC, which are not suitable in common planar lipid bilayers [4]. Here we show that the contact bubble method is suitable for a functional reconstituion of the smallest potassium channel $Kmpv_{12T}$ known so far. Surprisingly the channel shows an increased activity in the presence of membrane thickening lipids like PIP_2 or cholesterol.

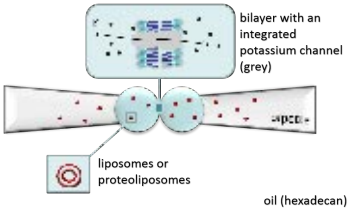

Figure 1: Two bubbles were blown in a bath of oil out of two pipettes filled with 100 mM potassium chloride, liposomes and potassium channels in nanodisks. At the oil-water interfaces monolayers can form. Bringing the bubbles into contact generates a bilayer and ion channels can integrate. [2]

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Cooperation of Local Motions in the Hsp90 Molecular Chaperone ATPase Mechanism

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The 90-kDa heat shock protein Hsp90 is a molecular chaperone that facilitates activation of a wide array of cellular "client" proteins essential for signal transduction, and is frequently implicated in cancer [1]. Hsp90 undergoes large domain rearrangements during its ATP-dependent chaperone cycle, resembling a molecular clamp that closes and opens. The role of critical local conformational changes within the machinery, which have been identified by crystallography, is elusive because there is a lack of suitable spectroscopic probes that can detect them in solution [2]. Here, we detected these motions by engineering probes based on contact-induced fluorescence quenching of an extrinsic label by the amino acid tryptophan through photoinduced electron transfer [3]. We found that the ATPase activity of Hsp90 was reflected in kinetics of lid-closure over the nucleotide binding pocket, domain-swap of the N-terminal β-strands and intra-subunit association of N- and M-domains. Nanosecond single-molecule fluorescence fluctuation analysis uncovered that the former two structural elements were highly mobile on the sub-millisecond time scale. Binding of nucleotide as well as of the activating co-chaperone Aha1 altered µs dynamics of the lid. The observation of burst phase kinetics in rapid mixing experiments supported a two-step mechanism of lid closure over the ATP-binding pocket and identified a previously unknown, early mode of activation of Hsp90 by Aha1. Results were published recently [4].

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The viral potassium channel KcvNH: a latent outward rectifier

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Viruses, which infect fresh water algae, code for very small membrane proteins with the structure and function of potassium channels (Kcv = K+-channel from Chlorella viruses). Even though these homotetrameric proteins have a monomer size of less than 100 amino acids, they show nearly the same electrophysiological characteristics as complex potassium channels. This simplicity makes them very good model systems for electrophysiological analysis. The previously uncharacterized Kcv-channel, Kcv_{NH} (Kcv New Hampshire), has a high sequence similarity with other known Kcv channels: Kcv_S (Kcv Smith) and Kcv_{NTS} (Kcv Next to Smith) [1] and Kcv_{ATCV-1} [2] (**figure 1A**). For electrophysiological characterisation of Kcv_{NH}, the patch-clamp technique in the whole cell conformation was used and showed an almost symmetric current response. Kcv_{NH} shows every characteristic of a functional potassium channel. Inward currents are blocked by Ba^{2+} and Cs^+. Replacement of K^+ with Na^+-ions leads to a negative shift of the reversal potential. Previous work [3] showed that the amino acid on position 77 has a high influence on the gating behaviour, controlling a voltage-independent slow gating process. Surprisingly, the Kcv_{NH} S77G mutant (**figure 1B**) showed a strong voltage dependence, visible as an outward rectification of its current response (**figure 1C**).

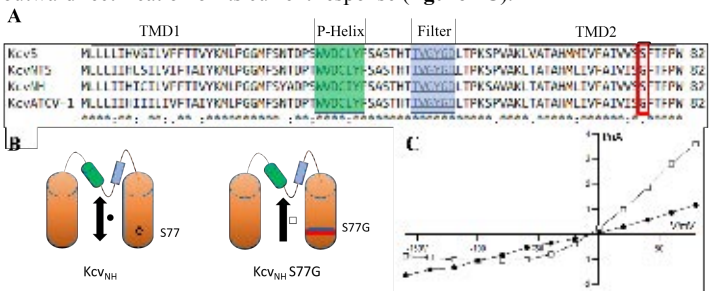

Figure 1: (A) Sequence alignment of the *Chlorella* virus channels Kcv_S, Kcv_{NTS}, Kcv_{NH} and Kcv_{ATCV-1}. (B) Schematic model of one monomer of Kcv_{NH} and the Kcv_{NH} S77G mutant. The amino acid position 77 is marked in each model (wildtype ◊, mutation in red). The direction of K^+-conductance is marked with a black arrow. (C) I/V diagram of Kcv_{NH} (●) and the Kcv_{NH} S77G mutant (□).

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Conformational changes of Channelrhodopsin-2 investigated by time-resolved EPR spectroscopy

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Channelrhodopsin-2 is a cation-selective light-gated channel[1], which is observed as a dimer. Its manifold usage has established channelrhodopsin-2 as the most prominent optogenetic tool[2]. Photoisomerization of the retinal leads to conformational changes of the protein, which open the channel. In contrast to other rhodopsins an outward movement of helix B during the functional process of the protein could be observed by distance measurements using pulse-EPR spectroscopy[3].

The photocycle, which describes the transitions between different intermediates of the protein upon light activation, is characterized by several time constants measured by time-resolved FTIR- and optical spectroscopy[3,4]. Mutants, which were available for distance measurements, were used here for time-resolved EPR spectroscopy. These method provides additional information about the relation of the transitions of the photocycle and the helix movements of the protein upon light activation.

The light-minus-dark difference continuous wave EPR spectrum with spin labels on helix B as well as helix F could be resolved and the respective time constant could be assigned to the last transition of the photocycle. Further, the origin of these difference spectrum could be explained simulating the cw EPR spectra by an increase of the mobility of the spin label. These results fit to previous results using pulse-EPR spectroscopy and show a transient outward movement of helix B[3].

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Infrared Reflection Absorption Spectroscopy: a Potent Method to Study Membrane Binding (Macro)molecules

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Infrared Reflection Absorption Spectroscopy (IRRAS) is a versatile technique to study the organization of interfacial films on a molecular or even sub-molecular level. It provides important information to understand macroscopic properties of interfacial layers. IRRAS combines the film balance technique, which allows to study thermodynamic properties of interfacial films with IR spectroscopy, which provides structural information, reveals the presence of molecules, their interactions, their conformation and their phase state. In addition, and in contrast to solution IR spectroscopy the orientation of molecules or molecular moieties can be determined by IRRAS. This is due to the perfectly flat geometry of the model system and the possibility to control polarization and incidence angle of the IR light.
This technique can be used to determine conformation and orientation in which fusion peptides bind to lipid membranes [1]. Furthermore, I will demonstrate how the orientations of membrane binding proteins can be determined and even structural rearrangements within large proteins during the binding process can be investigated in nearly natural conditions, i.e. physiological temperature, desired buffer composition and low protein concertation [2].

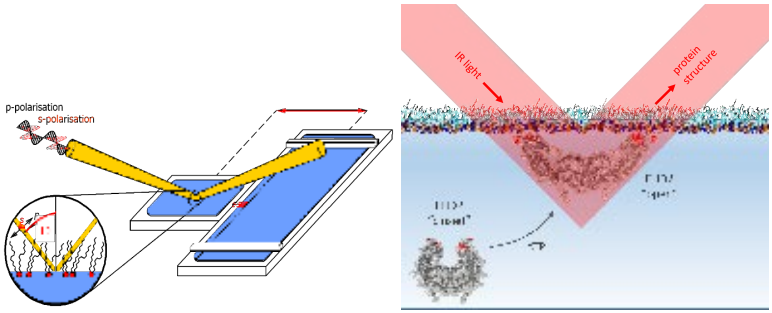

Figure 1: (left) Scheme of IRRAS setup (right) The caveolae stabilizing protein EHD2 adopted an open conformation upon binding to the lipid membrane, as determined by IRRA spectroscopy

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Lipid nanodiscs with genetically engineered MSP1D1 for structural and functional studies

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The function of a protein can be deciphered from its structural data. But structural studies of membrane proteins are difficult as these proteins have to be reconstituted into a native phospholipid-like environment to maintain their structure and function. Lipid nanodiscs present an advantageous membrane model system as they provide a native-like environment, are soluble, homogeneous and accessible from both sides of the reconstituted lipid bilayer. In addition, the low mass ratio of lipid to protein provides a suitable model system for future XFEL (X-ray Free Electron Laser) studies of membrane proteins for high-resolution structure determination without conventional crystallization. Nanodiscs are formed by the encircling of lipids by a Membrane Scaffold Protein (MSP). A genetically engineered MSP as in with cysteine modification will provide more possibilities for structural studies, the thiol reactivity can be used to form higher order structures, such as chains and arrays by disulphide linkage thereby creating a more ordered system for membrane protein structural studies. Apart from the structural study, it will also help in studying the topology of the system as such. Though a lot of membrane protein studies are carried out with nanodiscs, the topology of the system is still unclear. How do the MSP monomers align with respect to each other during formation of nanodiscs and what is the distance of separation between them or between their individual N- and C- termini? Fluorescence labelling of the Cysteines will help in answering the above questions through FRET analysis.

In this study, MSP1D1 wildtype and the three different engineered cysteine variants MSP1D1_N42C, MSP1D1_K163C and MSP1D1_N42C/K163C were purified for nanodisc preparation. The accessibility of the cysteines as attachment sites for chemical modification was confirmed by an N-1-Pyrene maleimide fluorescence assay showing that the cysteines are present in an aqueous environment and not buried in the lipidic phase. The introduction of FRET pairs using Atto dyes has been accomplished and will further help in understanding the topology and lipid-dependent dynamics of the nanodisc system.

Differential Ca^{2+}-sensing by GCAPs in rod and cone cells provide molecular basis of step-by-step regulation of retinal guanylate cyclase upon light activation

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Guanylate cyclases (GC) are key enzymes in many signaling pathways. In retinal phototransduction membrane bound GCs are regulated by small calcium sensors named Guanylate Cyclase-Activating Proteins (GCAPs). Upon light stimuli and fluctuating intracellular Ca^{2+}-levels, the GC-GCAP complexes regulate the cellular cGMP homeostasis thereby contributing to photoresponse recovery and light adaptation in photoreceptor cells.

The Ca^{2+}-dependent trigger of activity regulation in the GC-GCAP multiprotein complex is not understood at the molecular level. In fact, different experimental approaches yielded inconclusive results concerning the binding interface of GCAPs and target GCs. In the present study we investigated GCAP-GC interactions using a novel technique called Backscattering interferometry (BSI), which allows label- and immobilization-free interaction analysis at extremely high sensitivity. Here we show that GCAP1 and GCAP2 bind to its transmembrane target GC in a Ca^{2+}-independent manner. Furthermore, it will be demonstrated that the differential Ca^{2+}-sensing properties of GCAP1 and GCAP2 are reflected in two separate binding sites in the target GC. GCAP1 binds to the kinase homology domain near the trans-membrane region of the GC (i.e. juxtamembrane domain). Whereas GCAP2 did not interact with the GC in this region, an observation consistent with a model where GCAP1 and GCAP2 are permanently bound to GC at distinctly separated binding sites *in vivo*.

Finally, BSI proved to be an invaluable tool for the study of these previously intractable membrane targets, without the need of fluorescent labels and/or isolation of the target enzyme.

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Dissecting steps in ATP-driven protein translocation through the SecY translocon

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Most outer and inner membrane proteins in bacteria are transported and inserted by the ubiquitous Sec machinery (SecYEG complex) in the plasma membrane. Similar function is provided by Sec61 complex in the ER of eukaryotes. In prokaryotes, many proteins are only transported post-translationally via active translocation through the SecYEG at the expense of ATP hydrolysis by the peripheral membrane protein SecA and in concert with the trans-membrane proton motive force (PMF). Recently, using combination of biochemical, computational and single molecule techniques we have shed new light on the mechanism through which ATP hydrolysis is coupled to directional movement [1]. We combined all-atom molecular dynamics (MD) simulations with single molecule Forster resonance energy transfer (FRET) and biochemical assays and arrived at a new model for ATP driven protein translocation. In the model, ATP binding by SecA causes opening of the SecY-channel, by allosteric effect at site 5 nm away while substrates at the channel feedback to the active site and regulate nucleotide exchange. This two-way communication produces a 'Brownian ratchet' mechanism in which ATP binding and hydrolysis bias the direction of substrate diffusion and drive it across the membrane.
Using another FRET based assay with improved time resolution (0.1-100 ms time scale) we have also investigated individual steps in the transport, namely initiation and traslocation. This allowed us to estimate translocation rate at single molecule level. The initiation is rate limiting and depends on ATP binding and hydrolysis which in turn modulates conformational changes on millisecond time scale.
The section Molecular Biophysics of the German Biophysical Society organizes a meeting every other year in a small monastery in Hünfeld, close to Fulda, in the mid of Germany. Traditionally, this meeting is held as a joint meeting together with biophysicists from another European country, intended also to foster new collaborations or to strengthen existing ones between the two countries. Partners for 2017 are biophysical groups from Czech Republic.

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Interaction between HDAC6 and tubulin dimers/microtubules

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Human histone deacetylase 6 (HDAC6) deacetylates α-tubulin at Lys-40 and this post-translational modification is located lumenally in polymerized microtubules. As HDAC6 localizes predominantly in cytoplasm tubulin is the preferential substrate of the enzyme. Up to now knowledge of HDAC6 cellular functions is quite limited. Our efforts are aimed at the better understandingof molecular basis of the tubulin recognition by HDAC6. Here is shown kinetic analysis of tubulin deacetylation and interactions between HDAC6 and microtubules using individual purified components. From obtained data we show that free tubulin dimers are the preferred HDAC6 substrate with over 1,500-fold over tubulin polymers. Both longitudinal and lateral lattice interactions within polymerized tubulin forms are responsible for their the lower deacetylation rate when compared to free tubulin dimers. While studying HDAC6/tubulin interactions using TIRF microscopy we directly visualized stochastic interactions of HDAC6 with assembled microtubules without any preference for microtubule ends (Fig.1). When combined with previous studies on tubulin acetyltransferase (αTAT1) substrate specificity, our data provide explanation for the correlation between tubulin acetylation and microtubule age.

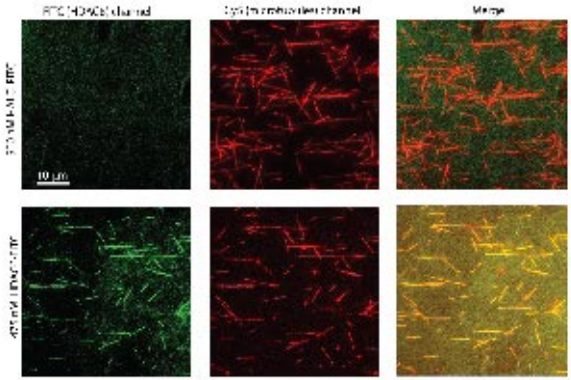

Figure 1: HDAC6 interaction with microtubules visualized by TIRF microscopy.
HDAC6 interacts directly with MTs. Cy5-labeled microtubules were immobilized on a glass coverslip surface. Micrographs are showing microtubules (red) in the presence of 500 nM HALO-FITC (green; negative control, upper panel) or 475 nM FITC-labeled HDAC6 (green; lower panel). In presence of FITC-labeled HDAC6, fluorescent signals of microtubules and HDAC6 co-localize along the whole length of microtubules (yellow).

Diisobutylene/maleic acid copolymer solubilizes membrane proteins into functional lipid-bilayer nanodiscs

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In vitro studies of membrane proteins are often hampered by the scarcity of suitable membrane mimics. Recently, styrene/maleic acid (SMA) copolymer has been shown to directly extract membrane proteins and their associated lipids from natural or artificial membranes to form functional polymer-bounded lipid-bilayer nanodiscs [1]. Biophysical characterization of these nanodiscs, however, has revealed enhanced dynamics of the lipid-bilayer core and restricted use of such polymers for enzyme assays involving Ca^{2+} or Mg^{2+}.

To address these limitations, we herein show that an alternating diisobutylene/maleic acid (DIBMA) copolymer performs on par with SMA in solubilizing phospholipids. It extracts proteins of various sizes directly from cellular membranes and stabilizes membrane proteins in functional bilayer nanodiscs. Most importantly, aliphatic DIBMA, in contrast with SMA, has only a mild effect on the lipid acyl-chain order and dynamics and is compatible with optical spectroscopy in the far-UV range as well as with functional protein assays involving divalent cations [2].

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K^+ channel selectivity filter gating correlates with site-specific ion occupation obtained from the same experiment

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The conformational transition between conducting and non-conducting states ("gating") in the selectivity filter of potassium channels is influenced by the occupation of the ion binding sites S0 – S4 inside the filter. This has been shown by numerous functional, structural and computational studies. However, which of the structural findings applies to which electrophysiological observation is not always clear. Here, we attempt to build a bridge between the different fields by the to-our-knowledge first identification of ion occupation and gating behavior from the same functional experiment by a combination of extended beta distribution analysis and IV curve modelling.

The viral K^+ channel Kcv_{NTS} serves a model system because of a strongly voltage-dependent gating process at negative potentials. Channels were expressed in vitro and reconstituted into planar lipid bilayers. The results show that the occupation of the outermost binding site S0 serves as the "voltage sensor" for the voltage-dependent gating in the selectivity filter of Kcv_{NTS}.

SOD1 as a novel sensor for in-cell protein folding experiments

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Though biomolecules function inside of living cells most biochemical assays are performed in dilute buffer solutions. These studies neglect the influence of the complex cellular environment on protein function and stability. Cells contain about 300 g/L macromolecules making the cellular milieu a highly crowded place [1]. Our group characterized the cellular crowding effects to understand how protein folding is modulated in the cell [2]. Here we developed a novel protein folding sensor to map how excluded-volume effects and non-specific interactions modulate protein folding stability inside cells. We used a truncated version of the protein superoxide dismutase 1 (SOD1) that showed reversible two-state folding in the test tube and the cell [3]. We studied a series of mutations of SOD1 that are related to the familial form of amyotrophic lateral sclerosis (ALS). Their stability was compared *in vitro* and in cells using Fast Relaxation Imaging (FReI) [4]. We found that most mutants were destabilized inside cells, however we found striking exceptions. Our approach allows to systematically study the role of protein surfaces to determine in-cell protein folding stability.

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