Biennial Meeting of the
# German Biophysical Society
**September 16–19, 2018**
**Düsseldorf, Germany**

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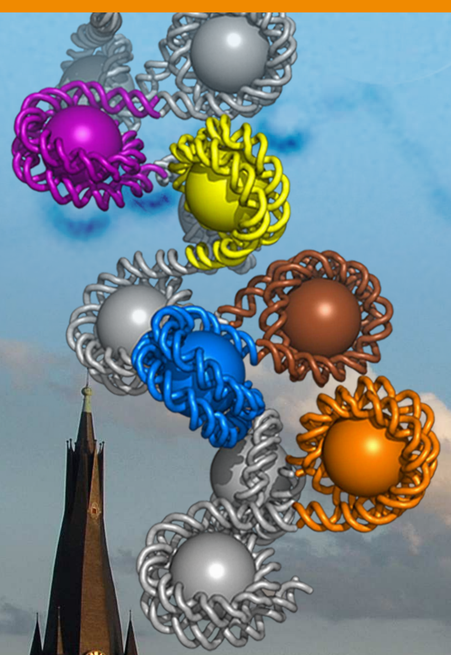
# Abstracts





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# Abstracts

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## Invited Speakers

### I-01. Rigorous analyses of allostery can focus the lens on dynamics of biomolecular systems

Harel Weinstein[1]

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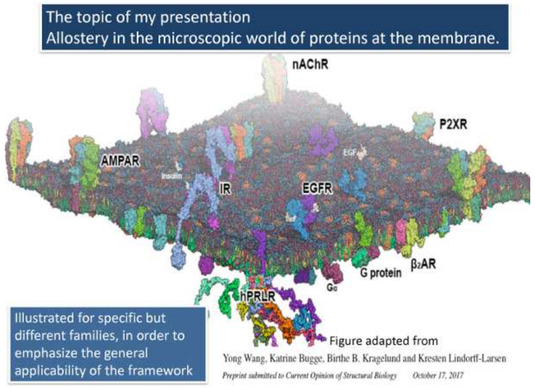
In performing their biological functions, membrane proteins transmit information with high fidelity. The transmission of information throughout molecular systems requires dynamic coupling between the conformations of discrete structural components within the protein positioned far from one another on the molecular scale. This type of biomolecular «action at a distance» is termed allostery. Because allostery is ubiquitous in biological regulation and signal transduction, its treatment in theoretical models requires rigorous quantitative descriptions involving the system»s underlying structural components and their interactions. We will illustrate the role of allostery in prototypical mechanisms of several membrane proteins in different functional classes (GPCRs, sodium-s ymport transporters, and lipid scrambling proteins) using analyses of very long timescale Molecular Dynamics simulations. These analyses employ formalisms we developed to obtain quantitative results that can reconcile biochemical and biophysical data about these systems through direct connection to molecular structure and dynamics.

Detailed methods, findings, and mechanistic conclusions to be presented, are documented in the following

Publications:
(1)- LeVine, MV, Weinstein, H - NbIT - a new information theory-based analysis of allosteric mechanisms reveals residues that underlie function in the leucine transporter LeuT. – PLoS Comput Biol 2014 10: e1003603; (2)- LeVine MV, Cuendet AM, Khelashvili G; Weinstein H - Allosteric Mechanisms of Molecular Machines at the Membrane: Transport by Sodium:Coupled Symporters. Chem Rev. 2016 116:6552;(3)- Razavi AM, Khelashvili G, Weinstein H - A Markov State-based Quantitative Kinetic Model of Sodium Release from the Dopamine Transporter. Nature Sci Reports 2017 7:40076; (4)- Cuendet AM, Weinstein H, LeVine MV - The Allostery Landscape: Quantifying Thermodynamic Couplings in Biomolecular Systems. J Chem Theory Comput. 2016 12(12): 5758; (5)- Morra G, Razavi AM, Pandey K, Weinstein H, Menon AK, Khelashvili G – Mechanisms of lipid scrambling by the G protein-coupled receptor opsin. Structure 2017 – Dec 18. pii: S0969-2126(17)30394-5; (6)- LeVine MV, Cuendet AM, Razavi AM, Khelashvili G; Weinstein H – Thermodynamic Coupling Function Analysis of Allosteric Mechanisms in the Human Dopamine Transporter. Biophys J. 2018 114:1–5; (7)-Terry DS, Kolster R, Quick M, LeVine MV, Khelashvili G, Zhou Z, Weinstein H, Javitch JA, Blanchard SC – A partially-open, inward-facing intermediate conformation of LeuT is associated with Na+ release and substrate transport. Nature Communications. 2018 9:230; (8)- Razavi AM, Khelashvili G, Weinstein H - How structural elements evolving from bacterial to human SLC6 transporters enabled new functional properties. BMC Biology 2018 Mar 14;16(1):3; (9)-Lee BC, Khelashvili G, Falzone ME, Weinstein H, Accardi A – Gating mechanism of the extracellular entry to the lipid pathway in a TMEM16 scramblase. Nature Communications 2018 – in press.

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## Invited Speakers

### I-02. The biophysics of RNP granule formation and disease

Simon Alberti[1]

[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Liquid-liquid phase separation can create functionally distinct reaction compartments consisting of proteins and RNAs called ribonucleoprotein (RNP) granules, which have major roles in cellular organization and physiology. RNA-binding proteins such as Fused in Sarcoma (FUS) are the key players that mediate the process of RNP granule formation by phase separation in cells. Recent data suggests that aberrant phase transitions of these proteins from a liquid to a solid-like state may be closely tied to the pathogenesis associated with diseases such as amyotrophic lateral sclerosis (ALS). Thus, elucidating how physiological phase separation gives rise to aberrant phase transitions and dysfunctional RNP granules will be key to understand these neurodegenerative diseases.

Here, we investigate how the prototypical RNA-binding protein FUS phase separates to form liquid-like RNP condensates that harden into less dynamic pathological structures. We find that phase separation is primarily governed by multivalent interactions among amino acid motifs that we call stickers. We show that these stickers are connected by flexible spacer sequences that govern the material properties of the condensates. We further demonstrate that the phase behavior of FUS in vitro critically depends on the RNA concentration: low RNA/protein ratios promote phase separation into liquid condensates, whereas high ratios prevent condensate formation. Moreover, reduction of RNA levels in cells causes excessive phase separation and the formation of pathological solid-like structures. Based on these data, we propose that phase separation is driven by a protein-intrinsic molecular grammar and that changes in RNA levels or RNA binding abilities of RNA-binding proteins cause aberrant phase transitions and disease.

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## Invited Speakers

### I-03. Pattern formation in cytoskeletal systems

Andreas Bausch[1]
[1]TU München, München, Germany

Living cells rely on the self-organization mechanisms of cytoskeleton to adapt to their requirements. In processes such as cell division, or cellular motility rely on the controlled self-assembly and disassembly of well defined active cytoskeletal structures interacting with lipid membranes. One important and promising strategy to identify the underlying governing principles is to quantify the underlying physical processes in model systems mimicking functional units of living cell. Here I»ll present in vitro minimal model systems consisting of active microtubule and actin filament systems which show pattern formation resulting from active transport processes. I will discuss how small variations in local interactions results in nematic or polar patterns in high density motility essays. With the example of reconstituted active vesicles I will discuss how to relate local force exertion and tension generation to shape transformations, blebbing, invagination or tethering of lipid membranes.

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## Invited Speakers

### I-04. Cells as actively responsive systems

Christine Selhuber-Unkel[1]
[1]CAU Kiel, Kiel, Germany

Cells are dynamic systems that actively interact with their environment. This interaction is not limited to chemical interactions, but also includes the sensing of mechanical and structural cues as well as the active application of forces and the restructuring of the environment by the cells themselves. A highly interesting question is therefore how cells sense, transduct and respond to forces. To investigate such aspects, single-cell force spectroscopy is an excellent tool, as cells can be tested while applying stimuli. In combination with dynamic interfaces, single-cell forces spectroscopy can be used to investigate the impact of mechanical stimuli. We have employed photoresponsive push-pull azobenzenes to apply an oscillatory stimulus to integrin receptors in fibroblast cells. The effect of such high-frequency molecular oscillatory forces on cell adhesion has been studied, demonstrating a significant reinforcement of cell adhesion in response to molecular oscillation. In the future, methods providing specifically designed cellular environments can provide strategies for controlling cells, also in biomedically relevant applications.

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## Invited Speakers

### I-05. More than just fat: Protein-lipid interactions in protein oligomerization and signaling

Rainer Böckmann[1], S. Gahbauer[1], S.A. Kirsch[1], A. Bochicchio[1], A. Sandoval-Perez[2], K. Pluhackova[3]

[1]Friedrich-Alexander University Erlangen-Nürnberg, Biology, Erlangen, Germany
[2]Universidad de los Andes, Bogota, Colombia
[3]ETH Zurich, Basel, Switzerland

The interaction of proteins and peptides with lipids is important for a wealth of biological processes, ranging from cell lysis, membrane fusion, transmembrane transport, to signaling and cell-cell communication. Proteins and lipids mutually influence their structure, localization, and mobility and thus function of each other.

The heterogeneity at the membrane/water interface plays a crucial role in the protein-lipid interactions: It yields a directionality, enables a stable anchoring of transmembrane proteins, and allows for specific targeting of peripheral proteins to distinct membrane domains. Here we report a novel hydrophobicity scale that allows to quantify the binding strengths of peptides to the membrane-solvent interface. The comparison of peptide toxicity and interfacial membrane binding energy reveals favorable peptide interactions with the headgroups of phosphatidylcholine lipids – the main component of eukaryotic plasma membranes – as the main driving force for the hemolytic activity of peptides.

Using multi-scale molecular dynamics simulations, we further show how a surface-attached coiled-coil [2], or membrane-embedded peptides [3,4] and proteins shape the membrane environment, i.e. induce membrane curvature, and bias the lipid structure and mobility. In turn, protein function is coupled to the lipid environment: Cholesterol may e.g. both induce as well as suppress specific GPCR dimers as discussed for different chemokine receptors [5,6]. Receptor oligomerization may thus be linked to membrane (nano)domain association of receptors as shown for the B cell receptor.

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## Invited Speakers

### I-06. Multi-color single-molecule FRET measurements investigate the real-time kinetics of multi-protein interactions in molecular machines

Thorsten Hugel[1]

[1]Albert-Ludwigs-Universität Freiburg i.B., Institute of Physical Chemistry, Freiburg i.B., Germany

Many molecular machines associate and dissociate dynamically and/or alternate dynamically between multiple conformations. Common techniques are not ideal for studying such dynamics on relevant time scales from sub-micro-seconds to several hours.

Here we show how multi-color single molecule FRET allows us to identify microscopic states in transient complexes of molecular machines. A correlation between conformational dynamics and nucleotide binding in the Hsp90 machinery is detected and quantified [1].

In addition, we solve the dynamic structures of the Hsp90 dimer in solution with a single-molecule FRET based approach [2]. The previously unknown open state of yeast Hsp90 is represented by an ensemble of conformations with inter-domain fluctuations of up to 25 Å. The fundamental precision and accuracy of single-molecule FRET measurements will also be discussed [3].

Finally, we present a novel plasmon ruler based single-molecule approach for studying the conformational dynamics of single molecules over 24 hours at video rate [4]. This opens new opportunities for studying heterogeneity among proteins, memory effects and directionality.

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[2] B. Hellenkamp, P. Wortmann, F. Kandzia, M. Zacharias, T. Hugel. Multidomain structure and correlated dynamics determined by self-consistent FRET networks. Nat Meth;14(2):174-180 (2017).
[3] B. Hellenkamp, S. Schmid, et al., Precision and accuracy of single-molecule FRET measurements - a multi-laboratory benchmark study. Nat Meth, in press.
[4] Ye et al., Conformational dynamics of a single protein monitored for 24 hours at video rate. arXiv: 1806.0715

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## Invited Speakers

### I-07. Phase separation and mesoscale assembly for functional compartmentalization

Tanja Mittag[1]
[1]St. Jude Graduate School of Biomedical Sciences, Memphis, United States

Liquid-liquid phase separation of proteins leads to demixing from solution and results in a dense, protein-rich phase, which co-exists with a light phase depleted of protein. Recent findings support a model in which phase separation is the biophysical driving force for the formation of membrane-less organelles in cells, such as stress granules, nucleoli and nuclear speckles. Current open questions are: (i) How is phase separation propensity encoded in the protein sequence, (ii) are dense liquid droplets used as reaction compartments in the cell, and (iii) is physiological phase separation disrupted in disease states? To address these, we study two systems, the tumor suppressor Speckle-type POZ protein (SPOP) and the RNA-binding protein hnRNPA1.

SPOP, a substrate adaptor of a ubiquitin ligase, localizes to different liquid membrane-less organelles in the cell nucleus, where it encounters its substrates, but it is never found diffuse in the cell. However, its recruitment mechanism to these organelles is not understood. Here, we show that SPOP undergoes LLPS with substrate proteins, and that this mechanism underlies its recruitment to membrane-less organelles. Multivalency of SPOP and substrate for each other drive their ability to phase separate. We present evidence that the SPOP/substrate assemblies are active ubiquitination compartments in vitro and in cells. SPOP cancer mutations reduce the propensity for phase separation. We propose that SPOP has evolved a propensity for phase separation in order to target substrates localized in membrane-less compartments.

Recent mutagenesis experiments have revealed the importance of aromatic residues for the ability of low-complexity regions (LCRs) of RNA-binding proteins to undergo LLPS. Here, we investigate the interactions that mediate phase separation of the intrinsically disordered LCR of hnRNPA1. Phase separation of hnRNPA1 promotes the fibrillization of mutants of hnRNPA1 that cause ALS and other neurodegenerative diseases. We find that aromatic side-chains cluster and lead to compaction of the LCR, and that this compaction is coupled to LLPS. Understanding the interactions that mediate phase separation has the potential to provide mechanistic insight into membrane-less compartmentalization in cells.

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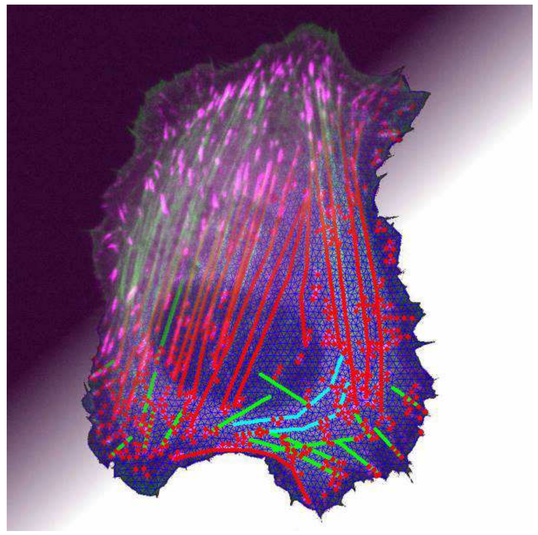
## Invited Speakers

### I-08. Active cell mechanics

Ulrich Schwarz[1]
[1]Heidelberg University, Department of Physics and Astronomy, Heidelberg, Germany

Biological cells use non-equilibrium processes to actively generate forces and movement. Some of these processes can be reconstituted in biomimetic experiments with active soft matter, nurturing the vision of a synthetic cell built from the bottom-up. I will first discuss how and why contractile forces are generated by non-muscle cells, and how these forces can be measured with traction force microscopy on soft elastic substrates. Surprisingly, quantitative cell experiments such as cell shape analysis, laser cutting or optogenetic control of cell mechanics reveal elastic properties for cellular systems that tend to flow on a molecular scale. I will discuss how these contractile systems can be mathematically described by continuum mechanics extended by active elements and how homogenization can be used to connect cell scale mechanics to the underlying molecular organization.



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## Invited Speakers

### I-09. Compley dynamics and dynamic complexes: NMR studies of large scale dynamics and thier role in protein function

Martin Blackledge[1], Nicola Salvi[1], Sigrid Milles[1], Serafima Guseva[1], Wiktor Adamski[1], Aldo Camacho-Zarco[1], Damien Maurin[1], Malene Jensen[1]

[1]Institut de Biologie Structurale, Grenoble, Germany

Proteins are inherently dynamic, exhibiting conformational freedom on many timescales,1 implicating structural rearrangements that play a major role in molecular interaction, thermodynamic stability and biological function. Intrinsically disordered proteins (IDPs) represent extreme examples where flexibility defines molecular function. IDPs exhibit highly heterogeneous local and long-range structural and dynamic propensities, sampling a much flatterenergylandscape than their folded counterparts, allowing inter-conversion between a quasi-continuum of accessibleconformations.2-4

In spite of the ubiquitous nature of IDPs, the molecular mechanisms regulating their interactions with physiological partners remain poorly understood. We use NMR spectroscopy, in particular exchange techniques such as relaxation dispersion and CEST, to map these complex molecular recognition trajectories at atomic resolution, from the highly dynamic free-state equilibrium to the bound state ensemble. Examples include the replication machinery of Measles virus, where the highly (>70%) disordered phosphoprotein initiates transcription and replication exploiting weak interactions with ordered and disordered domains of the nucleoprotein,5,6 the nuclear pore, where weak interactions between the nuclear transporter and highly flexible chains containing multiple ultra-short recognition motifs, facilitate fast passage into the nucleus.7 Finally, a combination of solution techniques reveals large-scale domain dynamics in the C-terminus of Influenza H5N1 polymerase that are essential for import into the nucleus of the infected cell.8

[1]. Lewandowski et al Science 348, 578 (2015)
[2]. Jensen et al Chem Rev 114, 6632 (2014)
[3]. Abyzov et al J.A.C.S. 138, 6240 (2016)
[4]. Salvi et al Angew Chem Int Ed. 56, 14020 (2017)
[5]. Schneider et al J.A.C.S. 137,1220 (2015)
[6]. Milles et al Science Advances 163, 734–745 (2018) In Press
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## Invited Speakers

### I-10. Water in biology

Martina Havenith[1]
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Exploring the unique role of water for life has been named as one of the top future challenges in chemistry. Whether fast protein motion and solvent dynamics are correlated at the very heart of enzymatic reactions is still under heated debate. Systematic studies under steady state conditions revealed that differences in the structure and thermodynamic properties of the waters surrounding the bound ligands are an important contributor. In order to decipher the role of water for biological function, such as enzymatic catalysis, novel experimental methods for water mapping, probing local entropy and enthalpy changes in real time are required. Terahertz (THz) spectroscopy is a new tool to probe changes in the low frequency spectrum of the solvated protein, which gives a direct access to the collective, (sub-)psec hydrogen bond dynamics, thereby opening a new window to directly probe the coupled protein/hydration dynamics. THz light extends the dielectric regime from nsec motions down to (sub-)psec motions, i.e. the timescale where librational, translational and intermolecular, collective motions of hydration water, as well as large amplitude motions of biomolecules, come into play.
Our group has developed linear and non-linear THz experiments to probe the change in hydrogen network dynamics in real time. Kinetic THz Absorption Spectroscopy (KITA) allows to record snapshots of the low frequency spectrum of a solvated proteins subsequent to initiation of the protein folding, thus capturing changes during hydrophobic collapse.
For ubiquitin and $\lambda$-repressor we were able to map the changes in the protein/solvent dynamics during protein folding of the cold denaturated protein with a time resolution of nsec.

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). We propose that the low frequency spectra of hydration water around solutes can be correlated with changes in entropy DS(t). In a proof of principle experiment on solvated alcohols we can show that this proposed correlation indeed holds [4]. THz calorimetry maps the solvent reorganization and will allow to record calorimetric properties far beyond equilibrium conditions.

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[2] T.Q. Luong, et al., Chemical physics letters 2016, 651, 1–7
[3] J. Dielmann-Geßner et al., PNAS 2014, 111 (50), 17857–17862
[4] F. Böhm, G. Schwaab, M. Havenith, Angew. Chem. Int. Ed. 2017, 56, 9981–9985

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### I-11. The molecular basis of Alzheimer's plaques: a near-atomic resolution structure of amyloid Aβ(1–42) fibrils

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Amyloid fibrils are associated with many diseases; amyloid-beta in particular is related to Alzheimer»s Disease. We present the structure of an Abeta(1-42) fibril determined by cryo-EM to a resolution of 4.0 Å. The fibril consists of two intertwined protofilaments in which individual subunits form a 'LS'-shaped topology revealing a complete new dimer interface compared to previous solid-state NMR models. All 42 aminoacids are well resolved in the EM density map, including the N-terminal part. The high resolution structure is in agreement with solid-state NMR and X-ray diffraction experiments.

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### I-12. Gaining traction: towards understanding the micromechanics of bacterial life

Benedikt Sabass[1]

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From developmental morphogenesis and tissue homeostasis to disease progression in cancer and infection by Gram-negative pathogens, cells must generate and coordinate mechanical forces. However, while mammalian cell mechanics has been an important focus of biophysical research during the last decades, the mechanobiology of bacteria is little studied. Here, the challenge essentially lies in quantifying dynamical processes that are beyond the optical resolution limit and linking them to complex, invisible molecular regulation processes. This requires advancing experimental methods, as well as quantitative modeling. In this talk, I first present a synopsis of methods and technological improvements that allow to measure mechanical processes at microscopic and nanoscopic scales. Next, I will discuss how measurement and simulation can be combined elegantly to study bacteria. Finally, I will present recent quantitative work on different bacterial species, highlighting fascinating, complex mechanical behavior and emergent phenomena.

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### I-13. In-situ investigation of outer membrane proteins in E. coli and native membranes using dipolar EPR spectroscopy

Benesh Joseph[1], Eva Jaumann[1], Arthur Sikora[2], Thomas F Prisner[1], David S Cafiso[2]

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[2]University of Virginia, Department of Chemistry and Center for Membrane Biology, Charlottesville, United States

Observing structure and conformational dynamics of membrane proteins at high-resolution in their native environments is a huge challenge for structural biologists. Here we describe a novel protocol for high-precision distance measurement in the nanometer range for outer membrane proteins (OMPs) in the native membrane and E. coli. OMPs in Gram-negative bacteria rarely have reactive cysteines. This enables in-situ labeling of engineered cysteines with a methanethiosulfonate functionalized nitroxide spin label (MTSL) with minimal background signals. Following overexpression of the target protein, spin labeling is performed with E. coli or the isolated outer membrane (OM) under specific conditions. The interspin distances are measured in-situ using Pulsed Electron-Electron Double Resonance (PELDOR or DEER) spectroscopy. The residual background signals, which is a serious problem for in-situ structural biology can be selectively removed to extract the desired interspin distance distribution. This approach provides a general strategy for observing protein-ligand interaction, oligomerization, and conformational dynamics of OMPs in native outer membrane and intact E. coli.

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### I-14. Shedding light on the molecular mechanisms of neuronal exocytosis: the primed SNARE-complexin-synaptotagmin complex

Axel Brunger[1]

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The central nervous system relies on electrical signals traveling along neurons at high speeds. Signals are also transmitted between two neurons, or from a neuron to a muscle fiber through synaptic junctions. Synaptic transmission relies on the release of neurotransmitter molecules into the synaptic cleft. This release in turn depends on a process called membrane fusion to ensure that the neurotransmitter molecules that are contained in synaptic vesicles are released into the synaptic cleft as quickly as possible. Membrane fusion is an important process in many areas of biology, including intracellular transport and hormone release, but it occurs much faster (< 1 millisecond) for synaptic vesicle fusion than for these other processes. Moreover, it is precisely calcium regulated. Recent structural and biophysical studies of the molecular mechanisms of neurotransmitter release will be presented.

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## Invited Speakers

### I-15. Structural basis for ligand recognition and signal transduction of GPCRs

Beili Wu[1]
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G protein-coupled receptors (GPCRs) comprise the largest protein family in human genome. The receptors sense molecules outside the cell and activate inside signal transduction pathways. GPCRs are involved in many human diseases, and represent the target of approximately 40% of all modern medicinal drugs. Structural studies of GPCRs remain enormously challenging. The human glucagon receptor (GCGR) belongs to the class B GPCR family and plays a key role in glucose homeostasis and the pathophysiology of type 2 diabetes. Here we report two crystal structures of full-length GCGR containing both extracellular domain (ECD) and transmembrane domain (TMD) at different conformational states. Notably, the stalk region, which connects the ECD and TMD, and the first extracellular loop (ECL1) undergo major conformational changes in secondary structure during peptide ligand binding, forming key interactions with the peptide. Hydrogen/deuterium exchange, disulfide cross-linking and molecular dynamics studies suggest that the stalk and ECL1 play critical roles in modulating peptide ligand binding and receptor activation. We further propose a dual-binding-site trigger model for GCGR activation, which requires conformational changes of the stalk, ECL1 and TMD. These insights into the full-length GCGR structure deepen our understanding about the signaling mechanisms of class B GPCRs.

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## T-01. Towards improved biophysical calculations to identify disease-causing mutations

Kresten Lindorff-Larsen[1]

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The explosion in sequencing of human genomes has revealed millions of missense variants that change protein sequences, yet we only understand the molecular and phenotypic consequences of a minute fraction of these variants. Equally important, we lack the mechanistic understanding necessary to develop therapeutic strategies for diseases caused by missense variants.

We have used in silico saturation mutagenesis, biophysical modelling and covariation-based calculations to predict the metabolic stability and thus functional levels of protein in cells. In a proof of principle study, we showed that missense variants in the MSH2 gene, known to cause hereditary cancer predisposition, lead to folding defects and rapid degradation in the cell. These are accurately identified by biophysical calculations and methods that examine sequence conservation and co-evolution.

We then assessed the generality of this mechanism, and selected 21 proteins associated with cancer, neurodegenerative and metabolic disease and different inheritance patterns. Our results show that disease-associated variants on average are substantially destabilized, while common variants in the population have little impact on stability. Overall, stability calculations successfully identified two-thirds of pathogenic variants.

Our calculations, however, also highlight the need for benchmarking and developing improved predictors of protein stability. We used high-throughput deep mutational scanning experiments, each assessing the effects of thousands of mutations, to benchmark and discover weaknesses in stability calculation methods. Many stability predictions methods work considerably better for hydrophobic deletion mutations than for mutations that increase the size of the amino acid, and other work poorly when using homology models. We have found that certain methods are able to overcome these issues, thus considerably expanding the applications in personalized medicine and biotechnological applications.

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## T-02. An optical zeptobalance for single-protein electrophoresis analysis

Mahyar Dahmardeh[1], Matthew P. McDonald[1], Vahid Sandoghdar[1]
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Ever since the original work of Arne Tiselius in the 1930»s, electrophoresis has been used as the gold standard assay for studying protein structures and proteomics. However, generally there are certain limitations inherent in the design such as the requirement for a large sample size, long experimental integration times and tedious post processing procedures. All of these make conventional electrophoresis techniques (i.e. slab-gel, capillary zone electrophoresis, etc.) arduous and in some cases impossible to implement.

Over the course of the last few years we have developed a label-free optical technique that senses individual nanoparticles and proteins using the interferometric detection of scattered light (iSCAT). iSCAT signals arise from interference between scattered waves and the light back-reflected from the microscope cover-glass surface. Since iSCAT operates by way of single protein optical detection, combining it with electrophoresis is advantageous not only in terms of overcoming the afore mentioned constraints, but also revealing more information about the diversity of proteins in the sample. In this regard, iSCAT only requires a concentration of a few ng/ml and can provide expeditious proteomic analyses.

We have thus developed an electrophoresis based technique compatible with iSCAT-based detection. Initial measurements indicated that this approach is viable and single proteins ranging from 24 – 460 KDa have been synchronously detected in a prototype iSCAT based apparatus. Utilizing microfabrication techniques, this work will seek to establish a novel method as a robust, easy-to-use procedure that can markedly supplement existing electrophoretic techniques.

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## Oral Presentations | Parallel-Session 1a – New Techniques

### T-03. Near-membrane refractometry and birefringence imaging of contractile HL-1 cells

Hossein Hassani[1], Andreas Offenhäusser[1]

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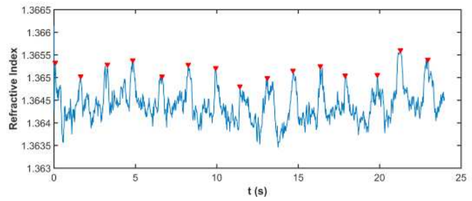
A reliable and high resolution information about the refractive index of the specimen, as a fundamental physical characteristic, is of crucial importance in many optical characterization techniques, especially when it comes to quantitative characterization of biological tissues. The relatively unexplored topic of cellular and subcellular refractive index and birefringence can help us harvest information about the structure and the function. Here we present an in-house built optical setup, capable of acquiring the local refractive index and birefringence in high spatial and temporal resolution and label-free manner. Contractile cardiac HL-1 cells have been studied using this method and the contraction signal has been successfully observed as momentary changes in the near-membrane refractive index. Furthermore, the refractive index imaging and birefringence imaging of these cells have revealed subcellular structure. As expected according to other studies reported in the literature, the refractive index of the cell nucleus was measured to be lower than that of the cytoplasm.

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### T-04. Cancer diagnosis and assessment of targeted breast cancer therapy by raman microscopy

Samir El-Mashtoly[1], Tatjana Frick[1], Hesham Yosef[1], Martin Schuler[1], Sascha Krauß[1], Axel Mosig[1], Klaus Gerwert[1]

[1]Ruhr-University Bochum, Biophysics, Bochum, Germany

Cystoscopy is the current gold standard for diagnosis of bladder cancer and it is an invasive method and painful for patients. Non-invasive diagnosis of urothelial carcinoma (UC) remains challenging. Urine cytology is the only non-invasive diagnostic method used in clinical practice and is used as an adjunct for cystoscopy. However, urine cytology has been criticized for its low sensitivity. Here, we present a non-invasive, label-free approach applied to urine using Raman spectral imaging and coherent anti-Stokes Raman scattering (CARS) imaging. Deep convolutional neural networks (DCNNs) were used to classify high-grade and low-grade urothelial cancerous cells with high accuracy. We also demonstrate that CARS microscopy has a potential application as a fast label-free imaging technique for pre-screening large amounts of cells in cytopathological samples.

Furthermore, we have investigated the distribution and metabolism of neratinib in breast cancer cells. Raman results indicated that neratinib has strong C≡N stretching vibration near 2208 cm-1, located in a Raman silent region of cells. Thus, it is used as a label-free marker band for neratinib. The Raman results also indicated that the drug in cells is metabolized. Two neratinib metabolites were identified using a combination of Raman microscopy, DFT calculations, and liquid chromatography-mass spectrometry. Thus, the above results show the potential of Raman microscopy as a non-invasive in vitro tool for cancer diagnosis and therapy evaluation.

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## T-05. Structural transformation of wireframe DNA origami via DNA polymerase assisted gap-filling

Thorsten Schmidt[1], Nayan Agarwal[1], Michael Matthies[1], Bastian Joffroy[1]
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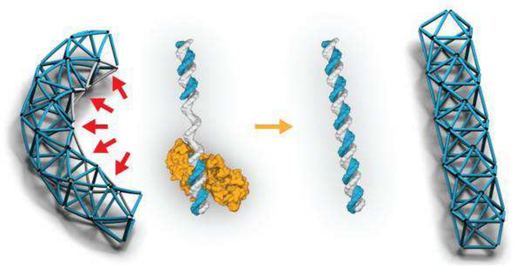
The programmability of DNA enables constructing nanostructures with almost any arbitrary shape, which can be decorated with many functional materials. Moreover, dynamic structures can be realized such as molecular motors and walkers. In this work, we have explored the possibility to synthesize the complementary sequences to single-stranded gap regions in the DNA origami scaffold cost effectively by a DNA polymerase rather than by a DNA synthesizer. For this purpose, four different wireframe DNA origami structures similar to our previous work were designed to have single-stranded gap regions. This reduced the number of staple strands needed to determine the shape and size of the final structure after gap filling. For this, several DNA polymerases and single-stranded binding (SSB) proteins were tested, with T4 DNA polymerase being the best fit.

The structures could be folded in as little as 6 min, and the subsequent optimized gap-filling reaction was completed in less than 3 min. The introduction of flexible gap regions results in fully collapsed or partially bent structures due to entropic spring effects. Finally, we demonstrated structural transformations of such deformed wireframe DNA origami structures with DNA polymerases including the expansion of collapsed structures and the straightening of curved tubes (Figure 1). We anticipate that this approach will become a powerful tool to build DNA wireframe structures more material-efficiently, and to quickly prototype and test new wireframe designs that can be expanded, rigidified, or mechanically switched. Mechanical force generation and structural transitions will enable applications in structural DNA nanotechnology, plasmonics, or single-molecule biophysics.



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## T-06. Viscoelasticity of cell membranes – from minimal artificial cortices to living cells

Andreas Janshoff[1]
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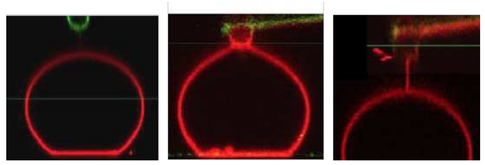
A thin layer of actin filaments located underneath the plasma membrane in eukaryotic cells drives pivotal aspects of cell mechanics. Myosin-driven contractility and actin-cytoskeleton membrane interactions are responsible for fundamental cellular processes such as cytokinesis, cell migration, cortical flows, and also contributes to the response to external deformation. How the interplay between the actin cytoskeleton, the membrane, and actin binding proteins drives these processes is far from being understood. To unravel general principles underlying actin cortex properties, a bottom-up in vitro system has been developed and compared with apical cell membranes derived from living cells.

By means of AFM indentation experiments and 2D microrheology employing video particle tracking we could show that essential features could be reproduced, while it is seemingly difficult to mimic the energy dissipation in real living systems upon deformation. We speculate that an intricate interplay between motor activity and dynamic cross-linking together with the presence of membrane reservoirs that buffer the tension in the cell membrane is responsible for this deviation between model systems and living cells.

The figure shows an AFM tip indenting a giant liposome and upon retraction pulling a tether.



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## T-07. Reconciling AFM and MD: unfolding focal adhesion kinase

Csaba Daday[1], Magnus Bauer[2], Maximilian Seidel[1], Pilar Redondo[3], Hermann Gaub[2], Daniel Lietha[3], Frauke Gräter[1]
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[2]Ludwig-Maximilians-Universität, München, Germany
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Molecular Dynamics simulations are routinely used to interpret single-molecule atomic force microscopy (AFM) or optical tweezer experiments. Most previous studies have focused on proteins with a relatively simple topology and their dissociation or unfolding events. When it comes to more complex proteins, the interpretation of force-extension curves can be challenging.

We here perform the direct comparison of the experimental and simulated unfolding pathway of focal adhesion kinase (FAK), a protein that, due to its complex three-dimensional topology, can unfold in numerous ways. FAK is a membrane-associated enzyme found in focal adhesions. In equilibrium, the kinase domain is inhibited by a so-called FERM domain, but is released under force [1].

We examine full FAK unfolding in both MD simulations and AFM experiments. We can experimentally identify the previously predicted [1] activation step in the first stage of the unfoldings. The further unfolding order depends on experimental conditions: in the presence of ATP, the kinase domain is stabilised and therefore the FERM domain unfolds first, and in the absence of ATP, the opposite is the case.

In the corresponding simulations, involving 1.5M atoms and 30 µs of simulation time, in the presence of ATP, the FERM domain indeed preferentially unfolds before the kinase, and this tendency is stronger at lower pulling velocities. Using our all-atom MD results, we successfully identify plausible sub-domain ruptures responsible for observed contour length increases in AFM.

We further extend our time scales by performing coarse-grained structure-based GoMartini [2] simulations. This allows for pulling velocities as low as 1 mm/s. While the unfolding pathways are similar to the all-atom counterparts, GoMartini does not fully recover the expected intermediates for this large and complex multi-domain protein.

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## T-08. The desmosome is mechanically loaded in response to externally applied stress

Anna-Lena Cost[1], Andrew J. Price[2], Alexander R. Dunn[2,3], Carsten Grashoff[1,4]

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[2]Stanford University, Biophysics Program, Stanford, United States
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[4]University of Münster, Department of Quantitative Cell Biology, Münster, Germany

Desmosomes are intercellular adhesion complexes that connect the intermediate filament cytoskeletons of neighboring cells and are essential for the mechanical integrity of mammalian tissues. Mutations in desmosomal proteins cause severe human pathologies including epithelial blistering and heart muscle dysfunction. However, direct evidence for when and even whether desmosomes bear load is lacking. To address this knowledge gap, we developed Förster resonance energy transfer (FRET)-based desmoplakin tension sensors to measure the piconewton-scale forces experienced by desmoplakin, an obligate protein that links the desmosomal plaque to intermediate filaments; we investigated forces across both major isoforms, desmoplakin I and desmoplakin II, in two complementary epithelial model systems. Our experiments reveal that neither desmoplakin I expressed in simple epithelia nor desmoplakin II expressed in basal keratinocytes experience significant tension under homeostatic conditions in cell culture, but desmoplakin becomes mechanically loaded in both model systems specifically when cells are exposed to external mechanical stress. The stress-induced loading of desmoplakin is transient and sensitive to the magnitude and direction of applied deformation, consistent with a stress absorbing function for desmosomes that is distinct from previously analyzed cell adhesion complexes.

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## T-09. Nanodroplets at lipid membranes undergo morphological transitions with spontaneously broken symmetry

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Lipid bilayer membranes exposed to different aqueous phases have been studied experimentally and theoretically. One example is provided by phase separation by polymer solutions within lipid vesicles[1] Such aqueous two-phase systems enclosed in lipid vesicles lead to partial to complete wetting transitions, vesicle budding, and vesicle tubulation. These processes are related to wetting of flexible substrates, and not yet completely understood. In the nucleation regime, the aqueous phase separation starts with the nucleation and growth of nanodroplets of one of the phases at the membranes, a process that is not accessible to optical microscopy. To elucidate the interactions between the nanodroplets and the membranes, we performed dissipative particle dynamics (DPD) simulations of a small liquid droplet of phase α in contact with the lipid membrane and surrounded by a different phase, β. The membrane is observed to engulf the nanodroplet to reduce the droplet»s interfacial area and, thus, its interfacial free energy. The final geometry of the membrane-engulfed droplets observed in the simulations is not axisymmetric and exhibits a non- axisymmetric membrane neck.[2] We show that this spontaneously broken symmetry is driven by a negative value of the line tension of the contact line between droplet and membrane.

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## T-10. A cell-topography based mechanism for ligand discrimination by the T-cell receptor

Kristina Ganzinger[1], Ricardo A. Fernandes[1], Justin Tzou[1], Peter Jönsson[1], Steven F. Lee[1], Matthieu Palayret[1], Ana Mafalda Santos[1], Alexander R. Carr[1], Aleks Ponjavic[1], Veronica T. Chang[1], Charlotte Macleod[1], B. Christoffer Lagerholm[1], Alan E. Lindsay[1], Omer Dushek[1], Andreas Tilevik[1], Simon J. Davis[1], David Klenerman[1]

[1]Max-Planck-Institut fuer Biochemie, Abt. Zelluläre und Molekulare Biophysik, Martinsried, Germany

The T-cell receptor (TCR) is a cell-surface protein that triggers the elimination of pathogens and tumors by T lymphocytes. To avoid causing damage to the host, the TCR must discriminate between di erent-potency
ligands presented by infected cells and tumors, but despite its immense importance for a functional immune response, exactly how the TCR does this is unknown. Here, I will present recent biophysical work showing that the formation of 'close contacts' between T-cells and model cell surfaces, characterized by spontaneous CD45 and kinase segregation at the submicronscale, initiates signaling even when TCR ligands are absent, suggesting that the receptor is activated by a local shift in the kinase/phosphatase ratio alone. I will also present unpublished work, in which we have now explored what makes TCR triggering both extremely sensitive and selective in these phosphatase depleted contacts { despite the fact that the mechanism is inherently ligand-independent and that agonist peptides are vastly outnumbered by non-agonist peptides. We have developed a new quantitative model of receptor triggering that allows calculations of the probability that receptor triggering is elicited at regions of contact between T cells and
target cells, following the local depletion of large, signaling-antagonistic tyrosine phosphatases. The model reveals that robust ligand discrimination is possible but that it requires the phosphatase-excluding contacts to be 400 nm in diameter or smaller, suggesting microvilli-mediated cell-cell contacts could function to suppress spurious T cell activation. Using a very limited number of experimentally-derived parameters the theory predicts ligand potency with remarkable accuracy, highlighting the role of surface topography in determining signalling outcomes. Altogether, this work provides a quantitative framework for understanding how immune responses start and for optimizing immunotherapeutic receptors.

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## T-11. Biomimetic light-harvesting funnels for re-directioning of diffuse light

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Over millions of years, nature has achieved a remarkable efficiency in harvesting diffuse light photons and directing them onto an energy-converting device, the photosynthetic reaction center. These processes occur in light-harvesting pigment protein complexes that consist of about 300 randomly oriented pigments funneling the energy of absorbed photons toward the reaction center via several ultrafast, very efficient energy transfer steps. The concept nature teaches us is based on efficient absorption of diffuse light, funneling excitation energy to special pigments, and directing them on very efficient charge separating units. Depending on the actual supply of solar photons, nature achieves close to unity efficiencies in converting photons into a primary charge transfer.

Efficient sunlight harvesting and re-directioning onto small areas has also great potential for more widespread use of precious high-performance photovoltaics but so far intrinsic solar concentrator loss mechanisms outweighed the benefits. Here we present an antenna concept allowing high light absorption without high reabsorption or escape-cone losses. An excess of randomly oriented pigments collects light from any direction and funnels the energy to individual acceptors all having identical orientations and emitting ~90% of photons into angles suitable for total internal reflection waveguiding to desired energy converters. This is achieved using distinct molecules that align efficiently within stretched polymers together with others staying randomly orientated. Emission quantum efficiencies can be >80% and single-foil reabsorption <0.5%. Efficient donor-pool energy funneling, dipole re-orientation, and ~1.5–2 nm nearest donor–acceptor transfer occurs within hundreds to ~20 ps. Single-molecule 3Dpolarization experiments confirm nearly parallel emitters. Stacked pigment selection may allow coverage of the entire solar spectrum.

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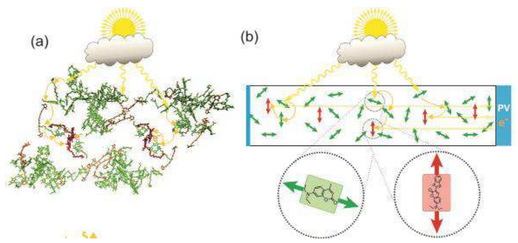


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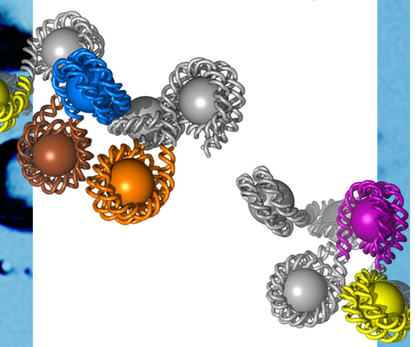



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## T-12. Complete kinetic FRET theory

Tobias Eilert[1], Eleni Kallis[1], Carl-Heinz Röcker[1], Jens Michaelis[1]

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The aim of FRET experiments is to obtain information about the proximity of objects at the molecular level. In many applications of FRET it is sufficient to prove a change in the efficiency in order to make statements about the systems. However, for the determination of e.g. 3-dimensional structures of macromolecules precise determination of distances from FRET measurements is requisite. The major obstacle here is that the fluorophores are attached to the macromolecules of interest by flexible linkers, because they introduce a lack of knowledge about the allowed positions and orientations of the dyes as well as their diffusional mobility. Although, the classic Förster theory describes the geometric effects on the transfer process exhaustively, the kinetic effects of the dye motions are not yet rigorously addressed.

We present a theory that treats the geometry and the kinetics of the FRET dyes as inseperably interacting factors which together determine the FRET efficiency. This and the stochastic nature of the dye motions and the FRET process itself led us to comprehend the FRET process as a stochastic phase space theory. This framework then allows us to derive general mathematical expressions for the average FRET efficiency by methods from statistical thermodynamics.

Our theory inter alia encompasses the approximately time-independent situations, the so-called limiting transfer regimes, and implicitly provides a manual for the computation by e.g. Monte Carlo integration. However, in case the transfer and the diffusion of the fluorophores happen on a similiar time scale our theory suggests a stochastic simulation in order to predict the FRET efficiency. The simulation is guided by parameters obtained by fluorescence lifetime and time-resolved anisotropy measurements. For dye molecules attached to dsDNA we find that the simulation produces results that are in quantitative agreement with experimentally determined smFRET efficiencies from experiments done on a TIRF microscope.

Additionally, the simulation allows us to study parameters which are usually hidden within the experiment. Here we show that for the example of dsDNA the usually applied kinetic assumptions are not justifiable over the common range of FRET efficiencies!

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## T-13. In silico screening for a bright circular permutation of red fluorescent protein

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Red fluorescent proteins are wildly well received tools to cell biologists, with the distinctive advantage of being genetically encoded, better biocompatible due to its lower phototoxicity associated with red-shifted visible light. Red fluorescent protein with fluorophore that»s unconventionally oriented and bright would be especially illuminating and can be applied as a key component in applications, such as Forster Resonance Energy Transfer (FRET) biosensors.

In the present study, we engineered a red fluorescent protein with an unconventionally oriented and bright fluorophore by first performing circular permutation on a newly developed red fluorescent protein mVermilion and then set up all-atom molecular dynamics simulations focusing on probing the relationship between the linker region and the micro-structure of fluorophore. In order to maximize its brightness, we focus on searching for a most planar and rigid fluorophore, a fundamental photophysical prerequisite for a bright fluorophore. The results show that the length and stiffness of the linker region has a direct tangible effect on the planarity of cpVermilion fluorophore, and thus its brightness.

Finally, the improved brightness of cpVermilion is confirmed in the experiment, which shows its brightness materially increased. The results here point to key role the linker region plays on the brightness of circularly permuted red fluorescent protein and in silico screening method can be adopted as an alternative to the established PCR based random mutagenesis screening method. And in silico screening for a most planar and rigid fluorophore could potentially be a general method for developing bright fluorescent protein.

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## T-14. DNA swelling as driving force of complex cellular functions

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DNA is classically considered as the material in which genetic information is stored. However, it becomes more and more evident that it is also an active material that affects cellular functions. Here, we show for a physiologically highly important process (NETosis) that biochemical reactions can drive DNA/chromatin out of equilibrium, which then causes drastic changes in structure and mechanics inside the cell.

Neutrophilic granulocytes are central protagonists of the innate immune system and provide defense against a variety of pathogens. They are able to release their own DNA as neutrophil extracellular traps (NETs) to capture and eliminate bacteria, fungi and viruses. DNA expulsion (NETosis) has also been documented for other immune cells as well as for amoebas and plant cells, thus highlighting the evolutionary conservation and importance of this process. Moreover, dysregulated NETosis has been implicated in many diseases, including cancer, vascular and chronic inflammatory disorders. During NETosis, neutrophils undergo dynamic and dramatic alterations of their cellular as well as sub-cellular morphology whose biophysical basis is poorly understood. We investigated NETosis in real-time on the single-cell level using fluorescence and atomic force microscopy. Our results show that NETosis is highly organized into three distinct phases with a clear «point of no return» defined by chromatin status. Entropic chromatin swelling is the major physical driving force that causes cell morphology changes and the rupture of both nuclear envelope and plasma membrane. Through its material properties, chromatin thus directly orchestrates this complex biological process.

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## T-15. Identifying benchmarks in hematopoietic stem cell adhesion and migration for nanometric variation of chemokine spacing versus synthetic agents

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In recent years, extensive research has been undertaken to relate the mechanical properties of a cell and its environment to specific cellular function. While standard genomic or proteomic techniques pioneered our understanding of the cell»s molecular architecture and signaling pathways, assessing the physical properties of cells is rising in popularity, since many of them are accessible without any cell modification.

Here, we explore hematopoietic stem and progenitor cell (HSPC) adhesion and migration on synthetic lipid membranes, displaying the chemokine SDF1α at nm-precise intermolecular distance. The synthetic membranes function as a model of the natural HSPC environment, the bone marrow niche, where stem cell growth and mobilization to the blood system is regulated. We characterize the complex interplay of HSPCs with the model niche, the cell adhesion and migration, for specific receptor-ligand pairs and quantify the impact of synthetic agents (e.g. drugs) on them. We use a combination of label-free microinterferometry and life cell imaging to monitor the cells, followed by extensive image data analysis. From the cellular tracks and shapes, we identify characteristic cell deformation pattern, in accordance with the underlying receptor-ligand distribution. Moreover, we introduce a simple theoretical model to describe HSPC migrating dynamics, which well reflects the corresponding cell deformation pattern. A comparison to primary leukemic blast cells shows that these malignant cells exhibit a significant decrease in cell mobility.

In conclusion, we show that the characteristic response of HSPCs to controlled ligand distribution is a powerful tool to decipher the physical mechanisms underlying HSPC (im-)mobilization from the bone marrow niche. With further systematic studies and complementary molecular information, a future benefit of this approach for leukemia therapy is envisaged.

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## T-16. Investigation of the kinetochore assembly by aberration-free and quantitative multi-color single-molecule localization microscopy imaging

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A key element for proper DNA segregation during mitosis is the kinetochore, a multi-protein complex that links the centromeric chromatin to the microtubules attached to the spindle pole body. [1] Our work aims at constructing a detailed map of the human-like regional kinetochore of Schizosaccharomyces pombe by single-molecule localization microscopy (SMLM).
For data acquisition, we set each kinetochore protein of interest (POI) into relation to two reference proteins (cnp1 at the centromere and sad1 at the spindle pole). This triple-color arrangement allows to triangulate the position of each POI and in time, to build a kinetochore model; assisted by visual analytics tools and resolved over the cell cycle at a nanometer resolution.
We rely on our recently developed mEos3.2-A69T[2,3] fluorescent protein variant, as it can be photoconverted by a novel mechanism using blue and infrared light, termed primed conversion (PC). This enables us to perform aberration-free multi-color imaging by combining mEos3.2-A69T with the UV-photoactivating protein PAmCherry to image two targets in the same spectral detection channel using the orthogonal PC and UV-activation modes.[3] The orientations of the complexes are determined by the conventional FP mScarlet-I, marking the spindle poles.
This way we determine protein stoichiometry, elucidate patterns in protein deposition and turnover, measure protein-protein distances. With this protein map we can visualize the assembly and regulation of the in situ kinetochore architecture for the very first time.

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## T-17. An integrative approach maps motions and conformers necessary for oligomerization of the large GTPase hGBP1

Thomas-Otavio Peulen[1], Carola S. Hengstenberg[2], Ralf Biehl[3], Mykola Dimura[1,4], Charlotte Lorenz[3,5], Alessandro Valeri[1], Semra Ince[2], Tobias Vöpel[2], Bela Fargo[6], Holger Gohlke[4], Johann P. Klare[7], Andreas Stadler[3], Claus A.M. Seidel[1], Christian Herrmann[2]

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[7]University of Osnabrück, Macromolecular Structure Group, Osnabrück, Germany

Guanylate binding proteins (GBPs) undergo a GTP controlled liquid-liquid phase separation to exert their biological function in mammalian cells - attacking intra-cellular parasites by disrupting the parasite»s membrane. Seeking for a molecular mechanism of GBP function, we applied small-angle X-ray scattering, fluorescence and electron paramagnetic resonance spectroscopy to study the human GBP 1 (hGBP1). We detected conformational heterogeneities which are resolved into structural models by integrative modeling. Combining neutron spin echo spectroscopy (NSE) with single-molecule FRET (smFRET) we probed for dynamics from nanoseconds to milliseconds and mapped hGBP1»s dynamics by a network of FRET-pairs. smFRET identified the C-terminus as flexible region. Based on these observations we propose that the flexible C-terminus ensures the formation of functional GBP oligomers and that GTP-triggered dimerization though GTPase-domains is followed by the association of C-termini. This suggests that intrinsic flexibility and «clocked» GTP-hydrolysis as the basic mode of action controlling GBP phase separations in living cells.

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## T-18. Molecular mechanisms in GTPase and ATPase proteins elucidated via experimental and theoretical FTIR and NMR spectroscopy, biomolecular simulations and CryoEM

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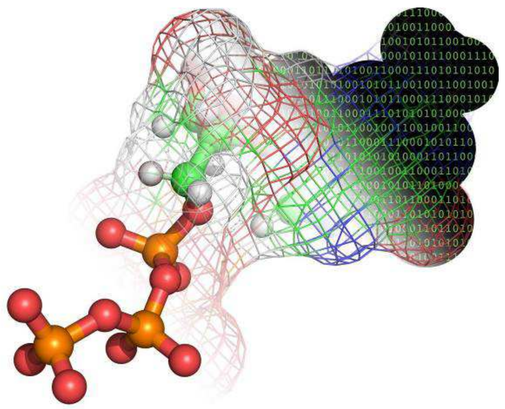
FTIR spectroscopy is a powerful method to elucidate protein mechanisms with high spatio-temporal resolution. In case of phosphate, 1 $cm^{-1}$ spectral change corresponds to a P-O bond length alteration of 0.001 nm. We utilized FTIR spectroscopy to characterize binding and hydrolysis of triphosphate compounds to GTPase and ATPase proteins. FTIR spectra were translated into 3D structural models using DFT QM/MM calculations. The result is a time-resolved movie of protein-catalyzed reactions with subatomic resolution.

The small GTPase Ras transmits signals in a variety of cellular signaling pathways, most prominently in cell proliferation. GTP hydrolysis in the active center of Ras acts as a prototype for many GTPases and is the key to the understanding of several diseases, including cancer. Therefore, Ras has been the focus of intense research over the last decades. A recent neutron diffraction crystal structure of Ras indicated a protonated γ-guanylyl imidodiphosphate (γ-GppNHp) group, which has put the protonation state of GTP in question. A possible protonation of GTP was not considered in previously published mechanistic studies. To determine the detailed prehydrolysis state of Ras, we calculated infrared and NMR spectra from quantum mechanics/molecular mechanics (QM/MM) simulations and compared them with those from previous studies. Furthermore, we measured infrared spectra of GTP and several GTP analogs bound to lipidated Ras on a membrane system under near-native conditions. Our findings unify results from previous studies and indicate a structural model confirming the hypothesis that γ-GTP is fully deprotonated in the prehydrolysis state of Ras.

We utilized this method ensemble supported with cryo electron microscopy to investigate the ABC transporters MsbA and MLA. Both are ATP powered transporters that maintain composition of the lipid membrane in cells.



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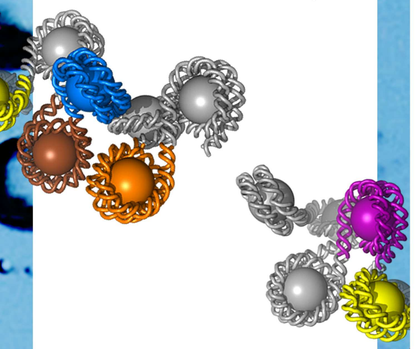



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## T-19. Biomolecular simulations for structural biology: integrating co-evolution, SAXS, fret at al.

Alexander Schug[1]

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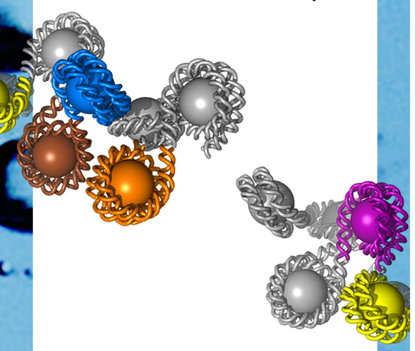
Exploring the interrelationship of structure and function is crucial for the understanding of molecular life. Yet despite significant progress of experimental methods, full characterization of functional cycles for proteins and RNA remains an ongoing challenge. Biomolecular simulations offer a complementary option to experiments. One can use such simulations akin to an atomically resolved microscope to gain insight into the dynamical motion of biomolecules.

It is also possible to include additional information as constraints into such simulations. The increasingly ubiquitous availability of sequential information and novel statistical inference methods have allowed to trace the co-evolution of residues [1], which can be exploited in structure prediction tools and is, e.g., sufficient for the blind prediction of proteins and RNA[2]. Similarly, one can use low-resolution experimental information such as SAXS data to model suitable biomolecular conformations [submitted] or compare data from simulations directly against experimental measurements such as smFRET data [3].

References
[1] Weigt M et al., Proc Nat Acad Sci USA (2009) 106, 67-72; Schug A et al., Proc Nat Acad Sci USA (2009); F. Morcos et al., Proc Nat Acad Sci (2011) 108, E1293-E1301
[2] Dago A et al., Proc Nat Acad Sci USA (2012), 109: E1733-42; E. De Leonardis et al., Nucleic acids research (2015); M. Figliuzzi et al., MBE (2016); G. Uguzzoni et al., Proc Nat Acad Sci USA (2017)
[3] I . Reinartz et al., J Chem Phys (2018)

Notes





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## T-20. Proton transfer across the protein fold is coupled to redox changes at the catalytic metal center of hydrogen-producing enzymes

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The global imperative to exit from nuclear power and fossil fuels demands exploring alternative “sources” of energy, e.g. biological H2. Hydrogenases are gas-processing metalloenzymes that catalyze H2 conversion in all domains of life. The so-called [FeFe]-hydrogenases show the highest rate of H2 release, with TOFs up to 10 kHz and negligible overpotential. Until today, the catalytic metal center of [FeFe]-hydrogenases inspired a plethora of H2-producing catalysts. The next generation of biomimetic compounds appears out of reach, however, without detailed understanding of the enzymatic proceedings.

Over the past three years, we developed a set-up to investigate hydrogenases and other gas-processing enzymes by in situ ATR FTIR spectroscopy. The diatomic ligands at the hydrogenase active site cofactor (i.e. CO and CN-) can be used as IR probes and facilitated a detailed description of protonation events and redox chemistry. Benchmarked by extensive 13CO isotope editing, DFT calculations were used to evaluate cofactor geometry beyond the limits of protein crystallography. Eventually, ATR FTIR spectro-electrochemistry indicated proton-coupled electron transfer (PCET) in the enrichment of certain redox species.

In the next step, in situ ATR FTIR spectroscopy was employed to follow protonation changes across the protein fold. Hydrogenases lack dedicated chromophores, thus H2 conversion activity was induced with a tailor-made redox dye. Upon reduction, the cofactor is rendered alkaline and takes up one proton from an adjacent amino acid. This triggers a “flip” of key residues in the conserved proton transfer pathway. Such mechanism was suggested by molecular mechanics simulations before, however our data represents the very first experimental evaluation of proton transfer dynamics in hydrogenases.

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## T-21. The amphipathic helix of Opi1 is fine-tuned to sense phosphatidic acid lipids in cellular membranes

Roberto Covino[1], Harald Hofbauer[2,3], Michael Gecht[1], Robert Ernst[4,3], Gerhard Hummer[1,3]

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[4]Saarland University, Homburg (Saar), Germany

The identity and function of cellular organelles is determined by the lipid composition of their membranes. In oder to survive, a cell must be able to sense and regulate this composition, based on its current metabolic needs and environmental challenges. Phosphatidic acid (PA) is the precursor for all other phospholipids and is predominantly found in the endoplasmic reticulum (ER) membrane. Depending on cellular needs, PA is either retrieved from lipid droplets or metabolized from sugars. In the baker"s yeast Saccharomyces cerevisiae, the soluble protein Opi1 tightly regulates the de novo synthesis of phospholipids based on the abundance of PA in the ER membrane, by either up- or down-regulating genes involved in lipid synthesis, storage, and retrieval pathways. Opi1 is therefore a PA membrane sensor that acts as a master gatekeeper of lipid synthesis.
Here, by using an integrated combination of in vivo and in vitro experiments, and extensive Molecular Dynamics (MD) simulations, we report on the mechanism of membrane recognition by Opi1 and identify an amphipathic helix (AH) for the selective binding to membranes containing PA over phosphatidylserine (PS). Based on these findings, we rationally design the membrane binding properties of Opi1 to control its responsiveness in the physiological context. Using atomistic MD simulations, we study the influence of PA on the binding, folding, and stability of the sensing AH on membranes. We identify two PA-selective residue motifs that tightly bind the phosphate headgroup, while interacting less intimately and more transiently with PS, showing that the AH of Opi1 is fine-tuned to recognise PA. This work establishes lipid headgroup selectivity as a new feature in the family of AH-containing membrane property sensors.

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## T-22. Maintenance of bacterial outer membrane lipid asymmetry: Insights from molecular simulations

Ulrich Kleinekathöfer[1], Shreyas S. Kaptan[1], Javier Abellón-Ruiz[2], Bert van den Berg[2]

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[2]Institute for Cell and Molecular Biosciences, The Medical School, Newcastle University, United Kingdom

A unique and very efficient permeation barrier to protect the cell from noxious compounds such as antibiotics if formed by the asymmetric outer membrane of Gram-negative bacteria. The asymmetry of the bilayer is due to phospholipids in the inner and lipopolysaccharides (LPS) in the outer leaflet and is crucial for the barrier function of the outer membrane. Although entropically favourable the presence of phospholipids in the outer leaflet causes enhanced permeation rates for many small molecules including some antibiotics and thus is potentially dangerous for the bacterium. The lipid asymmetry is ensured by the maintenance of lipid asymmetry (Mla) pathway which consists of six components. Until recently, the protein involved in the first step, i.e., MlaA, was structurally unknown and thus the molecular details of the respective mechanism which does not require external energy input. In a combination of X-ray crystallography and molecular dynamics simulations as well as in functional assays it was established that MlaA is a monomeric alpha-helical protein that functions as a phospholipid translocation channel. In molecular simulations it becomes apparent that MlaA with its donut shape is embedded in the inner leaflet of the outer membrane . Fully atomistic and coarse-grained simulations have been performed to unravel the mechanism of phospholipid extraction from the outer leaflet. Moreover, free energy calculations help us to understand the details of the transport from the outer leaflet towards the protein MlaC which in turn shuttles the phospholipid to the inner membrane.

J. Abellón-Ruiz, S. S. Kaptan, A. Baslé, B. Claudi, D. Bumann, U. Kleinekathöfer and B. van den Berg, Structural Basis for Maintenance of Bacterial Outer Membrane Lipid Asymmetry, Nat. Microbiol. 2, 1616–1623 (2017).

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## Oral Presentations | Parallel-Session 3b – Transporters and Channels

### T-23. Structural basis of membrane protein insertion via SecYEG translocon

Alexej Kedrov[1,2], Lukas Kater[2], Roland Beckmann[2]

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[2]Ludwig-Maximilians-University, Gene Center Munich, Munich, Germany

Integral membrane proteins form a large part of a cellular proteome, but they also require dedicated pathways for efficient targeting, insertion and folding. In spite of the recent developments in the structural analysis, molecular mechanisms of membrane protein biogenesis are remaining in focus of intensive discussions. SecYEG/Sec61 translocon is a universally conserved cellular machinery that facilitates the insertion of nascent membrane proteins. Here, we set out to elucidate the structure of the SecYEG:ribosome complex in the physiologically relevant environment of lipid-based nanodiscs by cryo-electron microscopy and to analyze the translocon dynamics and the insertion mechanism. Using the cryo-EM reconstruction we have succeeded to build the most complete model of the SecYEG that manifests a novel partially open conformation not observed in a detergent environment. Cryo-EM and chemical crosslinking further suggest that the nascent transmembrane domain is inserted into the lipid bilayer via the “lateral gate” of the translocon.

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## T-24. Atomistic Mechanism of Alternating Access in a Heterodimeric ABC Exporter

Lars Schäfer [1], Hendrik Göddeke[1], M. Hadi Timachi[2], Cedric Hutter [3], Laura Galazzo[2], Markus Seeger[3], Mikko Karttunen[4], Enrica Bordignon[2]

[1]Ruhr-University Bochum, Theoretical Chemistry, Bochum, Germany
[2]Ruhr-University Bochum, EPR Spectroscopy, Bochum, Germany
[3]University of Zurich, Institute of Medical Microbiology, Zurich, Switzerland
[4]Western University, Department of Chemistry and Department of Applied Mathematics, London, Ontario, Canada

ATP-binding cassette (ABC) transporters are ATP-driven molecular machines that couple ATP binding and hydrolysis in the nucleotide-binding domains (NBDs) to large-scale conformational changes in the transmembrane domains (TMDs), ultimately leading to the translocation of substrates across biological membranes. The precise nature of the structural dynamics of the large-scale conformational transition and the coupling of NBD and TMD motions are still unresolved. We combined all-atom molecular dynamics (MD) simulations with electron paramagnetic resonance (EPR) spectroscopy to study the mechanism of the dynamic conformational transitions underlying the functional working cycle of the heterodimeric ABC exporter TM287/288. Extensive multimicrosecond simulations in explicit membrane/water environment show how in response to ATP binding, TM287/288 spontaneously undergoes conformational transitions from the inward-facing (IF) state via an occluded (Occ) intermediate to an outward-facing (OF) state. The latter two states have thus far not been characterized in atomic detail. ATP-induced tightening of the NBD dimer involves closing and reorientation of the two NBD monomers concomitant with a closure of the intracellular TMD gate, leading to an occluded state. Subsequently, opening at the extracellular TMD gate yields the OF conformer. The obtained mechanism imposes NBD-TMD coupling by tightly orchestrated conformational transitions, between both the two domains and also within the TMDs, ensuring that the cytoplasmic and periplasmic gate regions are never open simultaneously to both sides of the membrane.

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## Oral Presentations | Parallel-Session 3b – Transporters and Channels

### T-25. Protein-induced spontaneous membrane curvature

Christoph Kluge[1], Rainer Böckmann[1]

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Among other functions, the (local) curvature of cellular membranes acts as a sorting mechanism for transmembrane proteins, e.g. by accumulation in regions of matching spontaneous curvature, as shown recently for the potassium channel KvAP and the aquaporine AQP0 by Aimon et al. However, the direction of the reported spontaneous curvature levels as well as the molecular driving forces governing the membrane curvature induced by the proteins could not be addressed experimentally. Here, using both coarse-grained and atomistic molecular dynamics (MD) simulations, we find matching levels of induced spontaneous curvature for the homologous potassium channel Kv 1.2/2.1 Chimera (KvChim) and AQP0 when embedded in unrestrained lipid nanodiscs. Importantly, the direction of curvature could be directly assessed from our simulations: KvChim induces a strong positive membrane curvature ~0.036 $nm^{-1}$ while AQP0 causes a comparably small negative curvature ~-0.019 $nm^{-1}$, in excellent agreement with experiments. Additionally, detailed analyses of protein-lipid interactions within the nanodisc revealed that the potassium channel shapes the surrounding membrane via structural determinants as well as by the dynamics of its voltage-sensing domains. The membrane stress emerging at the protein-lipid interface as well as induced asymmetries between the nanodisc lipid leaflets promote a protein-proximal spontaneous membrane curvature. In contrast, the water pore AQP0 displayed a high structural stability and an only faint effect on the surrounding membrane environment.

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## T-26. Site-specific ion occupation in the selectivity filter causes voltage-dependent gating in a viral $K^+$ channel

Indra Schroeder[1], Oliver Rauh[1], Ulf-Peter Hansen[2], Deborah-Desiree Scheub[1], Sebastian Mach[1], Gerhard Thiel[1]

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[2]Christian-Albrechts University, Structural Biology, Kiel, Germany

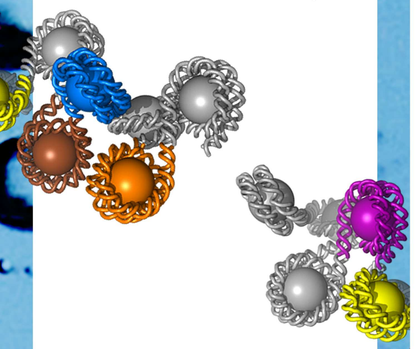
Many $K^+$ channels exhibit selectivity filter gating, often voltage-dependent and independent from dedicated voltage-sensor domains. A plethora of experimental and theoretical literature has shown that this is likely modulated by the occupation of $K^+$ binding sites within the selectivity filter. We approached this topic by analyzing single-channel gating kinetics and by revealing details on ion occupation from the same set of lipid bilayer experiments.

Kcv channels are viral pore-only $K^+$ channels; their selectivity filter gating, as observed from in vitro expressed proteins in planar lipid bilayers, is modulated by both voltage and $K^+$. This sub-ms gating is beyond the canonical resolution of the experiments and was thus analyzed by fitting extended beta distributions to amplitude histograms (1). A global fit of the rate constants and the single-channel IV curves based on available atomistic models of ion transport was performed for different voltages and $K^+$ concentrations. The rate constant of channel closing correlates with the probability of three ions being in the filter instead of two.

The experimental results further point to a crucial role of flexibility in modulating selectivity filter closure rather than a direct conformational change. The role of the inherent flexibility for gating in Kcv channels is currently further explored by anisotropic network modelling.

(1) Rauh, O., U.-P. Hansen, S. Mach, A.J. Hartel, K.L. Shepard, G. Thiel, and I. Schroeder. 2017. Extended beta distributions open the access to fast gating in bilayer experiments-assigning the voltage-dependent gating to the selectivity filter. FEBS Lett. 591:3850–3860. doi:10.1002/1873-3468.12898.

## Notes





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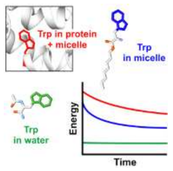
## T-27. Dissecting nanosecond dynamics in membrane proteins with dipolar relaxation upon tryptophan photoexcitation

Sandro Keller[1],Erik Frotscher[1], Georg Krainer[1,2], Michael Schlierf[2]

[1]Technische Universität Kaiserslautern (TUK), Molecular Biophysics, Kaiserslautern, Germany
[2]Technische Universität Dresden, B CUBE - Center for Molecular Bioengineering, Dresden, Germany

The structural dynamics of proteins on the nanosecond time scale can be probed with dipolar relaxation in response to photoexcitation of intrinsic tryptophan (Trp) residues. For membrane proteins, however, the complexity due to overlapping contributions from the protein itself, the membrane mimic, and the aqueous solvent impairs detailed analysis and interpretation. To disentangle these contributions, we measured time-resolved emission spectra of Trp in the protein Mistic in detergent micelles of various polarities. By comparison with Trp analogues in water and micelles, we could dissect the contributions from hydration, micelle, and protein matrix to dipolar relaxation on the nanosecond time scale. Our results demonstrate that ultrafast, subnanosecond relaxation reports on the extent of Trp shielding from water, with micelle and protein moieties making additive contributions. By contrast, relaxation in the low nanosecond regime is due to dipolar rearrangement of micelle and protein moieties upon photoexcitation, thereby probing conformational dynamics around the intrinsic fluorophore.

Frotscher et al. J. Phys. Chem. Lett. 2018, 9, 2241

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## T-28. Mapping vibrational energy transfer in peptides and proteins with non-canonical amino acids and ultrafast infrared spectroscopy

Erhan Deniz[1], Katharina B. Eberl[1], Jan G. Löffler[1], Tobias Baumann[2], Matthias Hauf[2], Fabian Schildhauer[2], Patrick Durkin[2], Nediljko Budisa[2], Jens Bredenbeck[1]

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[2]Technical University Berlin, Berlin, Germany

The understanding of allostery, in particular dynamic allostery (i.e. allosteric regulation without any major conformational change), is a field of increasing interest in the life sciences. Diverse theoretical studies suggest mechanically coupled networks of amino acids constituting signaling pathways to be linked to pathways of efficient vibrational energy transfer (VET). Experimental evidence for such distinct VET pathways, however, is still missing due to a lack of suitable methods.
Here, we show site-resolved VET measurements in tryptophan zippers (TrpZip) –short, well-structured peptides– and PDZ3, a domain of the postsynaptic density protein-95 and a paragon for dynamic allosteric regulation. We equipped both with the non-canonical amino acids β-(1-azulenyl)-alanine (AzAla) and azidohomoalanine (Aha), which we previously established as a protein-compatible VET donor-sensor pair.
Using ultrafast vis-pump IR-probe spectroscopy, we observe VET on a picosecond timescale between AzAla and Aha in the TrpZip and in the PDZ3-ligand complex. VET occurs via covalent bonds as well as non-covalent contacts (e.g. H-bonds). The VET timing nicely correlates with the donor-sensor distance and, so far, can be tracked over a distance of up to 17 Å.
Our experimental VET studies demonstrate that VET has the capacity to unveil both, covalently and non-covalently bonded amino acids constituting potential intra- and intermolecular signaling pathways across a protein for allosteric communication.

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## T-29. The mRNA 5'-untranslated region determines the helicase activity of the eukaryotic translation initiation factor eIF4A by modulating its conformational cycle

Dagmar Klostermeier[1], Alexandra Andreou[1], Ulf Harms[1]

[1]University of Münster, Münster, Germany

Translation initiation is the rate-limiting step of protein biosynthesis. In eukaryotes, translation initiation starts with binding of the eIF4F complex to the 5'-cap of the mRNA. eIF4F consists of eIF4E, which recognizes the cap, eIF4A, a DEAD-box RNA helicase, and eIF4G, a scaffolding protein that interacts with both eIF4E and eIF4A. The helicase activity of eIF4A is believed to mediate unwinding of RNA secondary structures in the 5"-untranslated region during scanning for the start codon. eIF4 is a special DEAD-box helicase that exists in three different conformations: an open conformation in the absence of ligands, a half-open conformation that is stabilized by eIF4G binding to both RecA domains of eIF4A, and a closed state in the presence of ATP, RNA, and the auxiliary factors eIF4G and eIF4B. eIF4B and eIF4G accelerate opening and closing of eIF4A, leading to a stimulation of ATPase and RNA unwinding activities, and a shift of the conformational equilibrium towards the closed state. Here we show that the RNA substrate itself also modulates the eIF4A conformational cycle, and thereby regulates ATPase and RNA unwinding activities. RNAs fall into two classes: Short single-stranded RNAs, or duplex RNAs with short 5'-single stranded tails do not elicit maximum stimulation of the eIF4A ATPase activity. In the presence of these RNAs, eIF4A is predominantly in the open conformation, and closing is rate-limiting for the conformational cycle. Longer single-stranded RNAs and RNAs with longer 5'-single-stranded tails, on the other hand, fully stimulate the ATPase activity of eIF4A. In their presence, the conformational equilibrium is shifted to the closed state, and conformational cycling is approx. 2-fold faster. In contrast, unwinding occurs with maximum velocity when the duplex is flanked by a short single-stranded tail. We present kinetic models for the catalytic cycle of eIF4A in presence of different RNAs, and show how the RNA substrate regulates its own unwinding.

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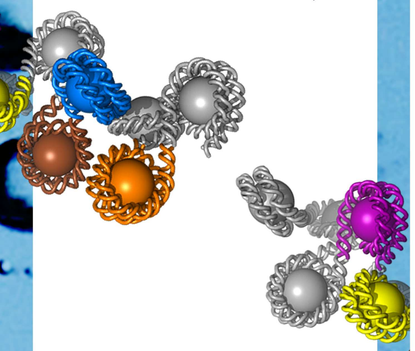



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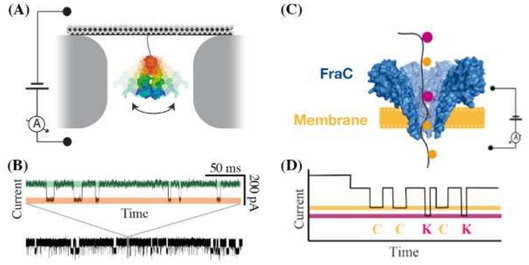
## T-30. High bandwidth sensing of single proteins with nanopores

Sonja Schmid[1], Laura Restrepo[1], Chirlmin Joo[1], Hendrik Dietz[2], Cees Dekker[1]

[1]Kavli Institute of Nanoscience Delft, TU Delft, Bionanoscience, Delft, Netherlands
[2]TU München, ZNN, Garching bei München, Germany

Electrical detection using nanopores provides several advantages over classical protein sensing techniques, one being the extensive temporal bandwidth from microseconds to hours. We exploit this to tackle the broad-range kinetics of proteins using a combination of solid-state nanopores with DNA-origami. Specifically, we anchor the protein of interest inside a nanopore (Fig. A) and monitor its behavior by means of conductance changes over time (Fig. B). This represents a radically new strategy to resolve the characteristic features of protein machines, namely conformational changes, as well as the binding, processing, and release of substrates & cofactors. We anticipate to detect broad-range kinetic effects that were previously overlooked due to narrow bandwidths.

In addition, we work towards protein fingerprinting (i.e. identification) using protein pores. To this end, we chemically couple specific amino-acids with artificial detection tags that were designed to cause distinctive current blockades (Fig. C, D). Comparing such “fingerprints” to an existing sequence database, we aim to ultimately quantify the protein content of a cell with single-molecule accuracy. Since the cellular protein composition - unlike the gene sequence - changes dynamically in response to disease, this information would herald an entirely new era in medical diagnostics.

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## Oral Presentations | Parallel-Session 4a – Biomolecular Dynamics

### T-31. Automated correlation-based structure refinement for high-resolution cryo-EM maps of large biomolecular complexes

Maxim Igaev[1], Carsten Kutzner[1], Lars V. Bock[1], Andrea C. Vaiana[1], Helmut Grubmüller[1]

[1]Max Planck Institute for Biophysical Chemistry, Theoretical and Computational Biophysics, Göttingen, Germany

We present a method for automated refinement of atomistic models into cryo-electron microscopy (cryo-EM) maps with resolutions reaching 2.5Å. The method rests on all-atom correlation-based molecular dynamics refinement with continuously adaptive resolution and simulated annealing. For several molecular systems of different complexity – from small well-behaved molecules to large complexes such as the ribosome – our method generates accurate atomistic models that agree better with the cryo-EM densities than the deposited reference structures. Simultaneous cross-validation against independent cryo-EM reconstructions during the refinement confirms that the improved quality is not due to overfitting. Overall, our method provides a fully automated and human-bias-free framework for efficient atomistic refinement of high-resolution cryo-EM data with widely accessible, moderate computational resources.

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## T-32. Relation between metastable oligomers and amyloid fibrils revealed by simultaneous analysis of their assembly kinetics

Wolfgang Hoyer[1,3], Filip Hasecke[1], Tatiana Miti[2], Daniel Schölzel[1,3], Lothar Gremer[1,3], Dieter Willbold[1,3], Philipp Neudecker[1,3], Henrike Heise[1,3], Martin Muschol[2]

[1]Heinrich-Heine-Universität, Physikalische Biologie, Düsseldorf, Germany
[2]University of South Florida, Department of Physics, Tampa, United States
[3]Forschungszentrum Jülich, ICS-6, Structural Biochemistry, Jülich, Germany

Deposits of protein aggregates consisting of amyloid fibrils with a characteristic cross-β sheet architecture are closely associated with a wide range of human disorders, including neurodegenerative diseases such as Alzheimer"s disease as well as non-neuropathic amyloidoses such as hereditary lysozyme amyloidosis. Apart from amyloid fibrils, the aggregation of amyloidogenic proteins frequently involves globular oligomers and their curvilinear assemblies referred to as protofibrils. Oligomers/protofibrils are long-lived metastable intermediates that have been suggested to constitute the main sources of cytotoxicity in amyloid diseases. Despite of their importance, the relationship between oligomers/protofibrils and amyloid fibrils has not been fully elucidated. Here we present the simultaneous analysis of the oligomer/protofibril and amyloid fibril assembly kinetics, employing fluorescence spectroscopy using the amyloid indicator dye Thioflavin T and atomic force microscopy. We examine two unrelated proteins, the amyloid-β peptide (Aβ) involved in Alzheimer's disease and lysozyme related to hereditary lysozyme amyloidosis, and find that both form metastable oligomers/protofibrils above a condition-dependent protein concentration threshold which we denote 'critical oligomer concentration'. The biphasic assembly kinetics reveal that metastable oligomer/protofibril formation is a reaction distinct from amyloid fibril nucleation (i.e., off-pathway), exhibits a far higher monomer concentration dependence, and interferes with amyloid fibril formation by (i) competing for the monomeric growth substrate and by (ii) active inhibition of amyloid fibril growth. Our results elucidate the interrelation between these two critical amyloid assembly types, which governs their temporal evolution and consequently their potential to exert specific toxic activities.

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## T-33. The auto-catalytic secondary nucleation of amyloid fibrils

Alexander Buell[1]

[1]Physical Biology, Biology, Düsseldorf, Germany

Amyloid fibrils are ordered, homopolymeric supramolecular assemblies of proteins, and a hallmark of many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. These structures form through a nucleated polymerisation mechanism and it has been shown that some amyloid fibrils are able to proliferate through an autocatalytic secondary nucleation mechanism, whereby the presence of amyloid fibrils catalyses the formation of more fibrils on their surface. This process has been proposed to be responsible for the production of toxic oligomeric and to play an important role in the sprading of disease pathology throughout the CNS of affected patients.

In this talk, I will summarise the evidece for the existence and importance of secondary nucleation in the case of the amyloid beta peptide and alpha-synuclein [1,2]. In addition, I will present the results of recent experimental and theoretical advances that allow to rationalise the dependence of the kinetics of secondary nucleation on factors, such as protein concentration [3] and temperature [4].

I will also present recent, unpublished data on the transmission of structural information by secondary nucleation.

References
[1] Buell et al., PNAS 2014, 111(21), 7671-7676
[2] Gaspar et al., Quart. Rev. Biophys. 2017, 50, e6, 1-12
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## T-34. Are gluten-related disorders a new protein aggregation disease? A biophysical approach to reveal the early stages of disease

Veronica I Dodero[1]

[1]Universität Bielefeld, Chemistry Department, Bielefeld, Germany

Gluten is a complex protein matrix present in wheat, rye, barley and some varieties of oats. In wheat, gliadin is the protein associated with gluten-related disorders, like celiac disease but also related to other diseases like autism. Gliadin is not fully degraded by humans producing an immunodominant fragment of 33 amino acid (33-mer) that triggers different immune responses in susceptible individuals. The cause and the early events that lead to the loss of its tolerance are not understood.

By a combination of spectroscopic and microscopic methods, we revealed that the 33-mer oligomerizes under physiological conditions forming different size oligomers and fibril-like structures. During the self-assembly process, a structural transition towards the characteristic amyloid β parallel structure occurs. Based on the structural and morphological similarities with other amyloid type diseases, we moved from chemistry and biophysics to immunological research, reporting that only large structures of 33-mer induce an innate immune response in macrophages mediated by Toll-like receptor (TLRs) 4.

Herein, I will present new insights into the characteristics of 33-mer superstructures obtained by cryo-TEM,and super-resolution optical microscopy. Our findings open a new understanding of the early stages of gluten-related disorders situating them, at least in vitro, as a new protein aggregation disease.

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## T-35. Multi-angle static and dynamic light scattering as a new in situ tool to investigate the self-assembly of proteins

Klaus Huber[3], Carlos Lopez[1], Oliva Sadanha[2], Thomas Witte[3], Sarah Köster[2]

[1]RWTH, Aachen, Germany
[2]University of Goettingen, X-ray Physics, Göttingen, Germany
[3]University of Paderborn, Chemistry, Paderborn, Germany

Formation of filaments by means of self-assembly of protein molecules attracts enormous interest in biophysical research as such processes lead to hierarchical structures highly relevant for cellular mobility and rigidity and transport processes within cells but also for disorders once misfolded proteins are prone to aggregate. This interest created a need for experimental techniques suitable to investigate such structure formation in situ. Light scattering turned out to be highly suitable, in particular if it is based on multi-angle detection systems. In an optimal case, combined static and dynamic light scattering (SLS and DLS) reveals the molar mass, geometric size, diffusion coefficient and shape of assemblies. However, the entire set of parameters can only be measured from growing assemblies once time-resolved experiments can be done with a series of detectors at variable angle simultaneously because only angular dependent data enable extrapolation of SLS and DLS data to zero scattering angle required for a proper evaluation of the parameters. The present work outlines two modern SLS and combined SLS/DLS multi-angle instruments1 and presents applications of this technique to the formation of beta-amyloid and vimentin. Whereas the former is correlated with Alzheimer"s disease, the latter is a major structural element in the cytoskeleton of animal cells that mechanically integrate other cytoskeletal components and absorb externally applied stress. The resulting in situ data could successfully be used to provide for the first time direct evidence for several features of the formation mechanisms which so far were only supported indirectly and/or based on invasive techniques. It thus extends our understanding of the assembly pathways of filament formation and its kinetics.

Lopez, C. G.; Saldanha, O.; Huber, K.; Köster, S. PNAS 2016, 113, 11152–11157.

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## T-36. From protein phase behavior to protein-protein interactions quantified by the second virial coefficient

Florian Platten[1], Jan Hansen[1], Dana Wagner[1], Stefan U. Egelhaaf[1]

[1]University of Düsseldorf, Soft Matter Physics, Düsseldorf, Germany

We experimentally determined the phase behaviour and the interactions of protein (lysozyme) solutions under conditions favouring protein crystallization and metastable gas-liquid phase separation. The phase behaviour as well as the interactions show striking similarities to those of colloids with short-ranged attractions. In particular, we find that the rescaled metastable gas-liquid binodals of protein solutions can be mapped onto those of square-well fluids. Despite their much more complex interactions, protein solutions hence appear to also follow the extended law of corresponding states.

Moreover, we exploit this extended law of corresponding states and apply the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory in order to infer the second virial coefficient $B_2$ from the phase behaviour, namely, the cloud-point temperature (CPT). This determination of $B_2$ yields values that quantitatively agree with results of static light scattering (SLS) experiments. Within this approach, the strength of the attractions is quantified in terms of an effective Hamaker constant, which accounts for van der Waals attractions as well as non-DLVO forces, such as hydration and hydrophobic interactions. This approach is based on simple lab experiments to determine the CPT and combined with the DLVO theory. Due to this simplicity, it can be applied to a wide range of protein-protein interactions including complex solution environments. This is expected to facilitate further biophysical research.

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## T-37. Studying the solution structure and function of the Yersinia type-III-effector YopO using an integrative structural biology approach

Gregor Hagelueken[1], Martin F. Peter[1], Caspar A. Heubach[1], Anne Tuukkanen[2], Fraser Duthie[1], Dmitri Svergun[2], Olav Schiemann[1],

[1]University of Bonn, Bonn, Germany
[2]EMBL, Hamburg, Germany

The bacterium Yersinia pestis is the causative agent of plague. To avoid the human immune system, they inject a set of six Yop proteins (Yersinia outer proteins) into attacking phagocytes. Inside the phagocyte, the Yop proteins interfere with several important cellular processes. One of these proteins, YopO, is subject of this study. Inside the host cell, YopO specifically interferes with the regulation of the actin cytoskeleton in various ways: 1) The C-terminus of YopO binds to Rac1, an actin cytoskeleton regulating GTPase and acts as a guanidine nucleotide dissociation inhibitor. 2) YopO binds to monomeric actin, forming a stable 1:1 complex. This interaction with actin leads to autophosphorylation and activation of the N-terminal kinase domain of YopO. 3) The bound actin molecule is then used as a bait to recruit and phosphorylate various cellular targets that are involved in cytoskeletal dynamics.
To investigate the molecular mechanism of actin induced YopO activation, we have conducted PELDOR distance measurements on both the spin labelled apo YopO and its actin complex. Overall, the PELDOR measurements are in agreement with a crystal structure of the YopO/Actin complex. However, they also indicate that compared to the complex, apo YopO must have a significantly different and more flexible structure. This was confirmed by small-angle X-ray scattering.
To produce a structural model of apo YopO, our rigid body docking software mtsslDock was extended such that PELDOR distance restraints and SAXS measurements can be simultaneously used as restraints during the docking procedure. In this way, we were for the first time able to deduce a structural model for the apo state of YopO. The apo structure gives important clues concerning the activation mechanism of this intriguing protein.

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## T-38. Molecular strain links to $Mg^{2+}$ dependent kinetic heterogeneity in a group II intron tertiary contact

Richard Börner[1], Fabio D. Steffen[1], Mokrane Khier[1], Danny Kowerko[2], Simon Gartmann[1], Roland K.O. Sigel[1]

[1]University Zurich, Chemistry, Zurich, Switzerland
[2]Chemnitz University of Technology, Department of Computer Science, Chemnitz, Germany

Metal ions are essential to mediate specific RNA-RNA interactions. These tertiary contacts stabilize the RNA fold and facilitate RNA catalysis such as in splicing where non-coding sequences (introns) are removed from the precursor mRNA. In a complementary way, RNA-DNA interactions allow mobile introns to reintegrate into new DNA sites. Numerous smFRET studies showed kinetic heterogeneity to be intrinsic to many RNA systems (1). Therein, partial or insufficient occupation of $Mg^{2+}$ binding sites has been discussed to be a major source of inhomogeneous kinetics for RNA-RNA contacts (2,3). However, the influence of specific metal ions on RNA-DNA interactions is still not fully understood. Here, we use smFRET in combination with molecular dynamic (MD) based Monte Carlo (MC) simulations as a hybrid approach (4,5) to investigate an RNA tertiary contact with known NMR structure (3). We explore the influence of $K^+$ and $Mg^{2+}$ on kinetic rates and stability of the RNA hairpin interaction with a complementary RNA or DNA sevenmer. In accordance with previous studies (2,3,5), we show that the RNA-DNA interaction displays a preference of $Mg^{2}$+ over $K^+$. A kinetic analysis indicates that the lower stability of the RNA-DNA complex results from an increased off-rate with respect to the RNA homoduplex. Interestingly, the RNA-DNA contact displays homogenous unbinding kinetics. Further, we use MD in combination with MC based FRET simulation and show that simulated and experimentally determined FRET values are in perfect agreement. We use MD to explore structural features and show that the different 3'- 2'-endo sugar pucker occupancies may alleviate molecular strain in the RNA-DNA contact with respect to the RNA homoduplex, which in turn is a potential source of the difference in kinetics (6).

References
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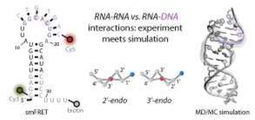
(2)PNAS (2015) 112(11):3403–3408.
(3) RNA (2014) 20(3):295–307.
(4) PONE (2011) 6(5):e19791.
(5) PCCP (2016) 18(42):29045–29055.
(6) Steffen et al (2018) to be submitted.

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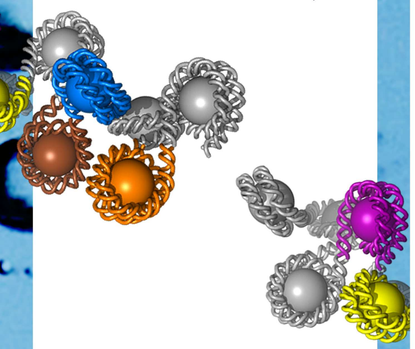



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## T-39. RNA processing and activation of type IIIA CRISPR-Cas systems

Brighton Samatanga[1], Gintautas Tamulaitis[2], Virginijus Siksnys[2], Ralf Seidel[1]

[1]Leipzig University, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany
[2]Vilnius University, Vilnius, Lithuania

CRISPR/Cas surveillance complexes are adaptive immune systems crucial in defence of bacteria and archaea against foreign DNA and viruses. CRISPR cassettes contain short conserved repeats interspaced by variable spacers. The spacers are integrated during viral infection and act as memory of the infection. Upon infection, the CRISPR loci are transcribed, and processed within the repeats, to produce small CRISPR RNAs (crRNAs). crRNAs are bound by Cas proteins to form CRISPR/Cas effector complexes that cleave foreign nucleic acids that are complementary to the crRNA guide. Main CRISPR/Cas systems are types I, II or III. While there is substantial data on the DNA recognition mechanisms of types I and II, the mechanism of RNA-dependent DNA cleavage by type III Csm/Cmr effector complexes is poorly understood. It is unknown how the Csm/Cmr complexes recognize RNA targets, and how this target binding activates DNA cleavage. RNA binding is immediately followed by RNA cleavage; leaving open the question on how RNA recognition temporally regulates DNA cleavage. DNA cleavage continues for long times after cleavage of the activating RNA. The verification mechanism of target from non-target RNAs (e.g. RNAs with mismatches) is also unknown. Since effector complexes of type I (Cascade) and Csm/Cmr share strong structural similarity signifying deep evolutionary relationship, we hypothesized that type III CRISPR/Cas systems apply target recognition mechanisms similar to type I; which include a directional RNA binding and verification of the integrity of bound RNA. By cross-correlation spectroscopy and single molecule fluorescence, we establish that Csm complexes bind RNA directionally and possess plasticity in target RNA recognition Further, Csm complexes differentially retained cleavage RNA fragments as mechanism to temporally regulate DNA cleavage. These results have significant implications in mismtach tolerance by type IIIA complexes and their functions beyond antiviral defence.

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## T-40. RNA as a complex polymer with coupled dynamics of ions and water in the outer solvation sphere.

Heiko Lammert[1], Ailun Wang[2], Udayan Mohanty[2], Jose N. Onuchic[1]

[1]Rice University, CTBP, Houston, TX, United States
[2]Boston College, Chemistry, Chestnut Hill, United States

RNA is only able to fold into compact structures in solution in the presence of counterions that compensate its negative charge. Notably $Mg^{2+}$ plays a crucial role in stabilizing the structures of folded RNAs. More recently, counterions have also been implicated in the functional dynamics of small folded RNAs. We study a foldable 58 nucleotide fragment of ribosomal RNA in solution using molecular dynamics simulations in explicit solvent and in the presence of realistic ion concentrations. Dynamics of the RNA and of associated ions and water are strongly coupled. We identify all the association sites for $Mg^{2+}$, $K^+$ and water near the RNA and determine the distributions of residence times. Chemical preferences reflect the competition between $Mg^{2+}$ and $K^+$ to associate with specific RNA binding partners. A joint principal component analysis combines the dynamics of the RNA with the occupations of ion association sites and reveals collective dynamics of the RNA with its coordination environment, including changes in ion binding linked to conformational motions of the RNA.

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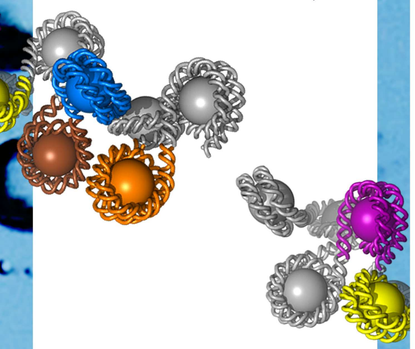



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## T-41. Temperature-dependet atomic models of detergent micelles refined against small-angle x-ray scattering data

Miloš Ivanović[1], Linda Bruetzel[2], Jan Lipfert[2], Jochen Hub[1]

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Surfactants have found a wide range of industrial and scientific applications. In particular, detergent micelles are used as lipid membrane mimics to solubilize membrane proteins. However, an atomic-level understanding of surfactants remains limited because experiments commonly provide only low-resolution structural information. MD simulations may provide atomic models of surfactant aggregates; however, MD simulations alone may be biased by force field imperfections. Therefore, we combined the strengths of SAXS with MD simulations to derive fully atomic models of two maltoside micelles (β-DDM and β-DM), at temperatures between 10°C and 70°C [1]. To this end, we coupled the simulations with an energetic restraint to the data, and we predict SAXS curves from the simulations using explicit-solvent calculations, thus employing accurate models of the hydration layer and excluded solvent [2,3]. First, we determined aggregation number as function of temperature using two independent methods, either based on the forward scattering with a model-free approach, or by comparing SAXS curves computed from MD simulations with the experimental data. The two approaches consistently show that aggregation number decreases with increasing temperature. Micelle models refined against SAXS data reveal that DDM and DM micelles are not prolate or oblate, but instead take the shape of a general tri-axial ellipsoid, where the major and middle axes decrease with increasing temperature, whereas the minor axis is nearly temperature-invariant between 10°C and 70°C. Notably, density profiles of water and hydrophobic groups along the micelle axes reveal that the minor axis closely mimics lipid membranes, with implications on the stability of protein-detergent complexes. This study highlights that a direct coupling between SAXS and MD simulation provides more spatially detailed and more reliable structures of soft matter systems, as compared to each of the methods alone.

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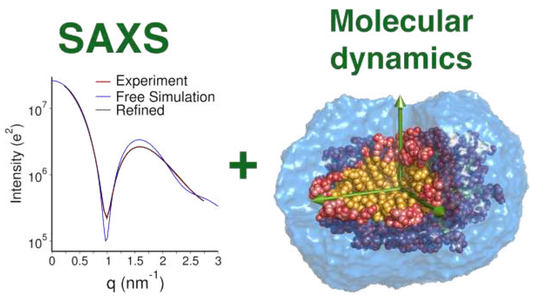


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## T-42. Immuno-IR-sensor identifies preclinical Alzheimer´s in blood

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Alzheimer"s disease (AD) is currently incurable, but there is general agreement that a minimally invasive blood-biomarker for screening preclinical stages would be crucial for future therapy. A hallmark in AD pathology is the misfolding and aggregation of Aβ. This misfolding is measured by a novel patented immuno-infrared sensor. The sensor is based on an ATR (Attenuated Total Reflection) element, which allows IR measurements of fluids containing water. In order to extract out of the blood the Aβ proteins, specific antibodies are bound to the ATR surface. In a discovery study of 10.000 people (ESTHER) and a validation study of about 1000 people (BiofFINDER) the test identified Alzheimer"s in preclinical states in blood with an accuracy of 88% about 8 years in average before clinical symptoms appear. In a two-stage approach using in a second step CSF and measuring again Aβ misfolding and in addition tau misfolding sensitivity and specificity far over 90% are obtained in a majority vote. The immuno-IR sensor can be miniaturised and further optimised towards a commercial application by using instead of an FTIR set-up the newest generation of Quantum-Cascade-Lasers.

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## T-43. Single amyloid fibrils studied in a thermophoretic trap

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One of the difficulties in studying protein aggregation into their fibrillar structures is the heterogeneity of the ensemble at all stages of the aggregation process. A mixture of monomers, oligomers and fibrils of various sizes determines the measurement outcome and commonly hides growth details such as secondary nucleation processes. Here, we try to remove this ensemble average by trapping a single fibril in a dynamic temperature field generated with the help of a plasmonic nano-structure. Our studies show that fibrils can be observed over time periods of at least several 10 minutes and a whole variety of properties can be accessed. In particular, the strong length dependence of the rotational diffusion provides a promising indicator for the real-time observation of the growth of a single fibril.

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## T-44. Fluid biomolecular condensates, their interaction with membranes and how they influence formation of autophagosomes

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Our knowledge about cellular functions has centered on two distinct types of compartments: the biological membrane and the cytosol. Now, fluid-fluid phase separation in the cytosol is increasingly recognized as a new major principle of cellular organization, whereby a novel type of compartment forms. These membrane-less condensates have fluid-like properties, they are not enclosed by lipid membranes and generate a new intracellular surface.

Autophagy is a complex intracellular pathway, important during development and implicated in severe diseases such as cancers and neurodegenerative disorders. The autophagosome, specialized double-membrane organelle generated de novo in the cytosol, leads to the membrane-bound degradation of cytoplasmic material. Fluid condensates, such as stress granules or P-bodies, are degraded by autophagsomes. This suggests that the interaction between the autophagic membrane and the surface of condensates play an important role during autophagy. Currently however, membrane-less condensates and membrane-bound organelles are regarded as separate entities and the contact between both is poorly understood.

Here, we address this central question. We report recent experimental and theoretical work analyzing the interaction between liquid compartments and membranes. By controlling assemblies precisely, we identified three distinct morphologies. Our theoretical analysis revealed several mechanisms of remodeling such as fluid scaffolding, not reported so far. We anticipate that our findings have direct implications for the general organization inside cells and as example, will discuss the mechanism of autophagosome formation at the surface of fluid compartments.

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## T-45. Transition networks for describing the disease related protein aggregation

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Many neurodegenerative diseases, such as Alzheimer's or Parkinsons disease, have as starting ground the abnormal aggregation of amyloid proteins, e.g. amyloid-β or α-synuclein, into oligomers and amyloid fibrils. Ever since the hypothesis that small oligomers are the toxic agents driving the disease and not the highly structured fibrils gained considerable ground, a lot of effort has been invested into studying the formation and structure of the small assemblies. Computational studies provide atomistic resolution models of oligomers and have proved so far indispensable to describing the aberrant aggregation process. For a better understanding of the early aggregation of amyloid peptides we have developed a novel analysis method of molecular dynamics simulations of aggregating peptides, based on aggregation transition networks. As applications we look at the early assembly of two short amyloid protein fragments into hexamers, the dimerization of the amyloid-β protein under different conditions, such as low pH, $Cu^{2+}$ ions and oxidation, and the assembly of the amyloid-β protein 1-42 into oligomers.

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## T-46. Predicting the structure of the γ-secretase - C99 complex

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γ -secretase is a membrane-spanning aspartyl protease known for cleaving single-pass transmembrane helices. Today, more than 90 γ-secretase substrates have been identified. Among these, the C-terminal fragment of the amyloid precursor protein (APP) C99, is the most thoroughly investigated - mainly because of its assumed role in the development of familial Alzheimer"s disease (FAD).

When processed by γ-secretase, C99 is cleaved several times, resulting in peptides of different length. Among other products, "amyloid beta" (Aβ) fragments Aβ40 and Aβ42 are formed. In healthy patients the 40 amino acid long Aβ40 peptide is the more abundant cleavage product of the two. This balance, however, can be shifted towards the longer Aβ42 fragment by various mutations. Aβ42 peptides that are assumed to be more prone to aggregation, have been heavily linked with the formation of amyloid plaques found in the brains of patients suffering from FAD.

Many aspects concerning C99 cleavage are still unclear: For instance, the nature of the substrate-receptor complex and the exact C99 binding region are still completely unknown and so is the mechanism leading to the different Aβ product lines.

By performing atomistic μs-timescale molecular dynamics simulations and free energy calculations, we compared three different γ-secretase-C99 complexes, representing the most widely believed C99 entry pathways. From these we were able to ascertain the most probable C99 binding site, enabling us to create a substrate-receptor model. This model explains the effect of many mutations and has allowed us to characterize key interactions between C99 and γ-secretase. Thorough understanding of C99 processing contributes substantially in the ongoing development of novel γ-secretase modulators, tackling the effects of adverse mutations.

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## T-47. The structure and dynamics of mutated amyloid β fibrils

Daniel Huster[1], Juliane Adler[1], Alexander Korn[1], Felix Hoffmann[2], Holger A. Scheidt[1], Anand Kant Das[3], Sudipta Maiti[3], Perunthiruti K. Madhu[3]

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[2]Martin-Luther University Halle-Wittenberg, Halle, Germany
[3]Tata Institute of Fundamental Research, Mumbai, Indonesia

A series of peptide mutants was studied to understand the influence of local physical interactions on the fibril formation mechanism of amyloid β (Aβ) (1-40). In the peptide variants, the well-known hydrophobic contact between residues $Phe_{19}$ and $Leu_{34}$ was rationally modified. In single site mutations, residue 19 was replaced by amino acids that introduce higher structural flexibility or restrict the backbone flexibility. Next, the aromatic Phe was replaced by aromatic residues to probe the influence of hydrogen bond forming capacity in the fibril interior. Also, charged residues were introduced to probe the influence of electrostatics. While the fibrillation kinetics and the local structure and dynamics of the peptide variants were influenced by the introduction of these local fields, the overall morphology and cross-b structure of the fibrils remained very robust against all the probed interactions. However, characteristic local structural and dynamical changes indicate that amyloid fibrils show an astonishing ability to respond to local perturbations but overall show a very homogenous mesoscopic organization. Interestingly, we find that even conservative mutations perturbing an early folding contact can drastically reduce the membrane-affinity and toxicity of Alzheimer"s Amyloid-β oligomers, without substantially affecting the end-state structure. Given such a significant biological importance of putatively minor sequence alterations, we currently focus on finding the minimally tolerated variation of the $Phe_{19}$-$Leu_{34}$ hydrophobic contact in Aβ(1-40). Further modification of $Leu_{34}$ and introduction of non-natural ring structures in position 19 lead to subtle differences in Ab toxicity confirming the importance of the $Phe_{19}$-$Leu_{34}$ contact in the pathology of Aβ peptides.

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## T-48. Cell free protein synthesis systems and single molecule fluorescence studies: A perfect marriage

Jörg Fitter[2,1], Alexandros Katranidis[1], Mayuri Sadoine[1], Michele Cerminara[1],

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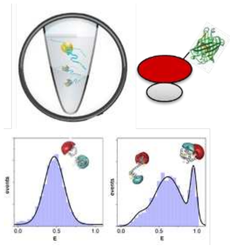
[2]RWTH Aachen University, I. Physikalisches Institut (IA), Biophysik, Aachen, Germany

Protein synthesis is a fundamental cellular process, by which ribosomes decode genetic information and convert it into an amino acid sequence. This highly complex process is accomplished by the translational machinery (ribosomes), accessory proteins, tRNA , mRNA and various factors. The fact that protein synthesis and translation does not necessarily require cell integrity, but can also proceed in so called cell-free protein systems, opens the door for comprehensive studies to obtain a deeper understanding of individual steps of the translation cycle and of the folding of de novo synthesized proteins. The use of cell-free protein synthesis (CFPS) systems allowed us to watch some of these essential these steps in real time and on single molecule level [1,2]. On the other hand the open nature of the CFPS system allows the production of protein samples perfectly suited for single molecule Förster resonance energy transfer (smFRET) studies. The strength of our CFPS based approach is given by the fact that we can accomplish either an direct incorporation of two different fluorescent dyes or the incorporation of reactive groups for a selective dye attachment at specific positions in the sequence [3]. Examples from both above mentioned topics will be presented demonstrating the strength of combining CFPS with single molecule fluorescence studies. [1] A. Katranidis, D. Atta, R. Schlesinger, K.H. Nierhaus, T. Choli-Papadopoulou, I. Gregor, M. Gerrits, G. Büldt and J. Fitter, Angewandte Chemie Int. Edit., 48, 1758-1761, (2009) [2] N. Kempf, C. Remes, R. Ledesch, T. Züchner, H. Höfig, I. Ritter, A. Katranidis and J. Fitter, Scientific Reports, 7, 46753, (2017) [3] M. Sadoine, M. Cerminara, N. Kempf, M. Gerrits, J. Fitter and A. Katranidis, Anal. Chem., 89, 11278-11285, (2017)



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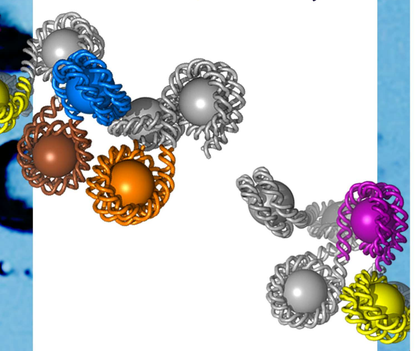



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## T-49. Light response of receptors within intact cells resolved by infrared spectroscopy

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Discrepancies between biophysical studies on isolated proteins and physiological studies in vivo may cast some doubt on the validity of results obtained in model environments. One prominent example are cryptochrome blue light receptors which bind flavin and regulate the growth and daily rhythm of plants. While some discrepancies between isolated receptors and cryptochrome mutants in plants concerning the photoreaction of flavin have been solved by EPR spectroscopy on cells, the actual protein response within cells remains an issue.

To perform in-cell spectroscopy, we first used the light-oxygen-voltage (LOV) domain in E. coli as a model system. Fluorescence spectroscopy was employed on the cells to investigate the kinetics of flavin recovery in LOV after illumination, which allowed us to verify the localization of LOV within the cells. FTIR spectroscopy in an attenuated total reflection configuration was established to record light-induced difference spectra on intact cells in suspension. The difference spectra revealed the characteristic signature of changes in secondary structure of LOV proteins demonstrating the integrity and full hydration of the proteins in our approach.

We then successfully extended the study to the light-sensitive domain of plant cryptochrome. The IR difference signature provided evidence for a light-induced β-sheet rearrangement of cryptochrome within the cells which is strongly stabilized as compared to the transient millisecond process in isolated receptors reported previously. Control experiments on isolated receptors allowed us to attribute this stabilizing effect to the binding of cellular ATP. We conclude that results from biophysical studies on isolated receptors were substantiated here by infrared spectroscopic studies on intact cells, including the stabilization of structural rearrangements into the range of physiological lifetimes.

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## T-50. New insights into molecular membrane dynamics from super-resolution microscopy

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Molecular interactions are key in cellular signalling. They are often ruled or rendered by the mobility of the involved molecules. We present different tools that are able to determine such mobility and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution. We depict how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS). We highlight how STED-FCS can reveal novel aspects of membrane bioactivity such as of the existence and function of potential lipid rafts.

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## T-51. A receptor tyrosine kinase study at the single-molecule level

Marina S. Dietz[1], Marie-Lena IE Harwardt[1], Phoebe Young[1], Hartmut H. Niemann[2], Mike Heilemann[1]

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Receptor tyrosine kinases (RTK) regulate various processes such as cell proliferation and motility. Dysregulation of RTK signaling pathways leads to diseases such as cancer [1,2]. MET is an RTK that is also the target of the pathogenic bacterium Listeria monocytogenes, which uses the receptor to invade epithelial cells with the surface protein internalin B (InlB) [3].

For a better understanding of receptor signaling and disease dysregulation, we used single-molecule and other fluorescence techniques and investigated resting and InlB-activated MET for oligomerization, dynamics, and association with cellular structures. We found preformed MET dimers in the absence of an activating ligand [4], determined the binding affinities of InlB to MET in vitro and in cells [4,5], and excluded artifacts that could arise by labeling the ligand with a fluorophore. We applied single-molecule tracking to investigate the dynamics of MET in living cells. By using different ligands and cytotoxins, we created a mechanistic model of MET receptor activation and its linkage with specific endocytosis pathways [6].

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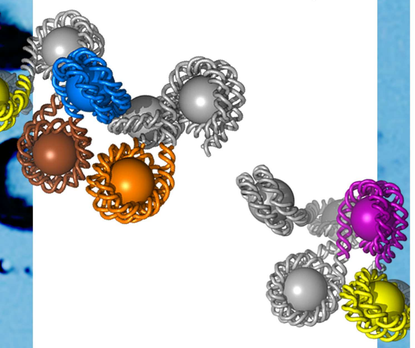



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## T-52. Studying membrane proteins and drug responses in individual breast cancer cells using liquid-phase electron microscopy

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Most knowledge about membrane proteins has been obtained via biochemical methods but these analyze pooled material from many thousands of cells so that the information is based on popula-tion averages. Moreover, the proteins are extracted from their native environment of the cell mem-brane. Examining membrane protein function is now possible for endogenous proteins (no genetic engineering required) at the single molecule- and single cell level in intact mammalian cells using liquid-phase scanning transmission electron microscopy (STEM) [1]. The key step is to specifically label the proteins of interest with small nanoparticles, for example, quantum dots. Hydrated cells are then placed in a defined liquid environment inside the vacuum of the electron microscope and im-aged with STEM. Another option is to cover the cells with a thin sheet of graphene protecting the liquid from evaporating [2].

Liquid-phase STEM was used to explore the formation of HER2 homodimers at the single-molecule level in intact SKBR3 breast cancer cells in liquid state [3]. HER2 is a membrane protein and plays an important role in breast cancer aggressiveness and progression. Data analysis based on calculating the pair correlation function from individual HER2 positions revealed remarkable differ-ences of its functional state between rare- and bulk cancer cells with relevance for studying the role of cancer cell heterogeneity in drug response. Liquid STEM was then used to explore the effect of the antibody-based drug trastuzumab at the single molecule level, thereby unraveling the molecular mechanism immediately after drug binding [4].

The authors thank E. Arzt for his support through INM, the DFG (SFB1027), and the Else Kröner-Fresenius-Stiftung.

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## T-53. A new paradigm for biomolecular interactions: ultrahigh-affinity complex of two intrinsically disordered proteins involved in chromatin remodeling.

Alessandro Borgia[1], Madeleine Borgia[1], Katrine Bugge[2], Vera Kissling[1], Pétur Heidarsson[1], Catarina Fernandes[2], Andrea Sottini[1], Andrea Soranno[3], Daniel Nettels[1], Birthe Kragelund[2], Robert Best[4], Benjamin Schuler[1]

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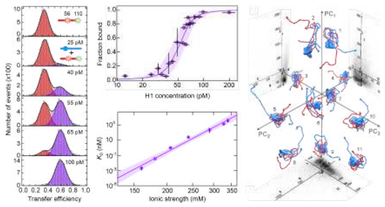
Interactions between biomolecules form the basis of biological communication on the molecular level. In the traditional paradigm of Biology, specificity and affinity are encoded in the precise geometry of complementary shapes at biomolecular interfaces. However, there is mounting evidence that many proteins involved in cellular interactions are unfolded under physiological conditions or contain large unstructured regions. These “intrinsically disordered proteins” (IDPs) may bind without folding, but the universal assumption has remained that at least one of the binding partners needs to form a binding site with well-defined structure, in order to achieve an affinity and specificity compatible with cellular function.

The human linker histone H1.0 is a highly positively charged protein involved in chromatin compaction by binding to nucleosomes, and is largely unstructured. The human nuclear protein prothymosin α (ProTα), is a fully unstructured acidic IDP also involved in chromatin remodeling and associated with transcription and cellular proliferation. ProTα has been reported to be a linker histone chaperone in live cells by interacting with H1 and increasing its mobility in the nucleus. Thanks to a combination of single-molecule fluorescence spectroscopy techniques, closely integrated with NMR and molecular simulations, we were able to characterize this binding reaction and have discovered an unexpected phenomenon: these two IDPs associate in a complex with picomolar affinity, but they fully retain their disorder and flexibility, defining a new paradigm of protein-protein interactions.

Importantly, the lack of a structure and highly dynamic character of the complex allows near-free access to post-translationally modifying enzymes and enables a much quicker transfer of H1.0 to and from nucleosomes: This could be the key to achieve fast access to DNA without compromising nucleosomal stability, with far-reaching implications for chromatin dynamics regulation.

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## T-54. Dissecting two differential binding mechanisms of FG-nucleoporins and nuclear transport receptors

Piau Siong Tan[1], Iker Valle Aramburu[1], Davide Mercadante[2,3], Swati Tyagi[1], Aritra Chowdhury[1], Daniel Spitz[1], Sarah L Shammas[4], Frauke Gräter[2,3], Edward A. Lemke[1,5,6]

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[4]University of Oxford, Department of New Biochemistry, Oxford, United Kingdom
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Phenylalanine-glycine rich nucleoporins (FG-Nups) are intrinsically disordered proteins, constituting the selective barrier of the nuclear pore complex (NPC), which can be crossed by nuclear transport receptors (NTRs). How the interaction of NTRs affects conformational features of FG-Nups to orchestrate the nucleocytoplasmic transport remains unclear. Currently a view is emerging that NTRs interact with FG-Nups via the formation of an archetypal-fuzzy complex, in which the conformational ensemble of the unbound FG-Nup is ready to bind a NTR without undergoing a substantial conformational change. These FG-Nup·NTR interactions are highly dynamic; multiple minimalistic low affinity binding FG-motifs engage with different NTR binding pockets with ultrafast kinetics. Such a binding mechanism can explain how fast yet specific transport of molecules across the NPC is possible [1]. In this work, we used single-molecule spectroscopy studies combined with atomistic molecular dynamics simulations to reveal that FG-Nup214, which localizes to the cytoplasmic side of the NPC, undergoes a clear and distinct conformational change when binding the CRM1•RanGTP export complex. This indicated that the formation of FG-Nup214•CRM1•RanGTP complex follows a coupled reconfiguration-binding mechanism which is different from the archetypal-fuzzy FG-Nup·NTR mechanism previously seen. We suggest that such a binding mechanism can regulate the spatial localization of export complexes to help cargo and NTR undocking processes [2]. Altogether, our study sheds light on the diverse dynamics of FG-Nups and brings us a step forward to understand the diverse mechanistic functions and regulatory role of different FG-Nups.

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## T-55. Probing the movements underlying activation and inactivation in voltage-gated K+ channels using fluorescent unnatural amino acids

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[1]Université de Montréal, Department of Pharmacology and Physiology, Montreal, Canada
[2]Université de Montréal, Department of Physics, Montreal, Canada

Excitability in heart and nervous system is based on sensing and propagation of the membrane potential by voltage-gated ion channels. Despite availability of high-resolution structures of voltage-gated ion channels, key questions about their activation and inactivation mechanisms remain elusive. We followed the movements of the gating machinery of the Shaker-Kv channel by introducing a fluorescent non-canonical amino acid along the linker between voltage sensor and pore and follow their movement by simultaneous electrophysiological and fluorescence measurements (voltage clamp fluorometry). Using two-color voltage-clamp fluorometry, we compared movements of the voltage sensor, along the covalent link to the pore, and the cytosolic gate with charge displacement and pore opening. We found that the proximal S4-S5 linker moves with the S4-helix throughout the gating process, while the distal portion undergoes a separate motion related to late gating transitions. Both pore and S4-S5 linker undergo rearrangements during C-type inactivation. In presence of accelerated C-type inactivation, the energetic coupling between movement of the distal S4-S5 linker and pore opening disappears. The data allow us to describe a complete model for the sequence of voltage activation.

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## T-56. The membrane activity of the fungal peptide toxin Candidalysin allows insights into the pathogenicity mechanism of the clinically relevant Candida albicans

Christian Nehls[1], Julia Wernecke[1,2], Laura Paulowski[1], Mareike Lewke[1], Helena S. Fabritz[1], Annette Crezelius[1], Christian Borchert[1], Julian R. Naglik[3], Bernhard Hube[4], Thomas Gutsmann[1]

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[2]Deutsches Elektronen-Synchrotron, Hamburg, Germany
[3]King´s College London, Dental Institute, London, United Kingdom
[4]Hans Knöll Institute, Department of Microbial Pathogenicity Mechanisms, Jena, Germany

For decades, researchers have been trying to elucidate critical determinants of the pathogenicity of human fungi. Only recently, has an international consortium of scientists succeeded in identifying such a factor – a peptide toxin secreted by the facultative pathogenic fungus Candida albicans. This peptide Candidalysin plays a crucial role during fungal infections of human mucosae. The peptide"s virulence manifests in the direct damage of epithelial membranes, in the stimulation of a danger response signaling pathway and in the activation of epithelial immunity. We provided the first insights into the direct interaction between Candidalysin and lipid membranes.

The peptide"s amphiphilic alpha-helical structure is described as a prerequisite for its binding to lipid membranes. In consequence of the initialized binding, the peptide inserts between the lipid head groups and aligns parallel to the bilayer surface. Upon increasing surface accumulation, however, the helix starts to penetrate the bilayer with oblique inclination. As a result, the bilayer is destabilized and transient local collapses occur. This carpet-like disintegration eventually leads to the disruption of the entire membrane. A comparison of host, bacterial and fungal model membranes will be used as an approach for understanding the selectivity of Candidalysin for non-self membranes. The influence of peptide aggregation and of C-terminal truncation on the mode of action of Candidalysin will be discussed.

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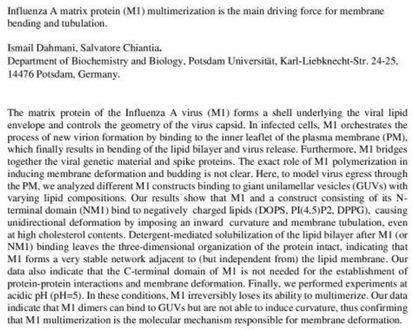
## T-57. Influenza A matrix protein (M1) multimerization is the main driving force for membrane bending and tubulation.

Ismail Dahmani[1,]

[1]Potsdam-University, Biochemistry , Potsdam, Germany

The matrix protein of the Influenza A virus (M1) forms a shell underlying the viral lipid envelope and controls the geometry of the virus capsid. In infected cells, M1 orchestrates the process of new virion formation by binding to the inner leaflet of the plasma membrane (PM), which finally results in bending of the lipid bilayer and virus release. Furthermore, M1 bridges together the viral genetic material and spike proteins. The exact role of M1 polymerization in inducing membrane deformation and budding is not clear. Here, to model virus egress through the PM, we analyzed different M1 constructs binding to giant unilamellar vesicles (GUVs) with varying lipid compositions. Our results show that M1 and a construct consisting of its Nterminal domain (NM1) bind to negatively charged lipids (DOPS, PI(4,5)P2, DPPG), causing unidirectional deformation by imposing an inward curvature and membrane tubulation, even at high cholesterol contents. Detergent-mediated solubilization of the lipid bilayer after M1 (or NM1) binding leaves the three-dimensional organization of the protein intact, indicating that M1 forms a very stable network adjacent to (but independent from) the lipid membrane. Our data also indicate that the C-terminal domain of M1 is not needed for the establishment of protein-protein interactions and membrane deformation. Finally, we performed experiments at acidic pH (pH=5). In these conditions, M1 irreversibly loses its ability to multimerize. Our data indicate that M1 dimers can bind to GUVs but are not able to induce curvature, thus confirming that M1 multimerization is the molecular mechanism responsible for membrane deformation.



Influenza A matrix protein (M1) multimerization is the main driving force for membrane bending and tubulation.

Ismail Dahmani, Salvatore Chiantia.
Department of Biochemistry and Biology, Potsdam Universität, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany.

The matrix protein of the Influenza A virus (M1) forms a shell underlying the viral lipid envelope and controls the geometry of the virus capsid. In infected cells, M1 orchestrates the process of new virion formation by binding to the inner leaflet of the plasma membrane (PM), which finally results in bending of the lipid bilayer and virus release. Furthermore, M1 bridges together the viral genetic material and spike proteins. The exact role of M1 polymerization in inducing membrane deformation and budding is not clear. Here, to model virus egress through the PM, we analyzed different M1 constructs binding to giant unilamellar vesicles (GUVs) with varying lipid compositions. Our results show that M1 and a construct consisting of its N-terminal domain (NM1) bind to negatively charged lipids (DOPS, PI(4,5)P2, DPPG), causing unidirectional deformation by imposing an inward curvature and membrane tubulation, even at high cholesterol contents. Detergent-mediated solubilization of the lipid bilayer after M1 (or NM1) binding leaves the three-dimensional organization of the protein intact, indicating that M1 forms a very stable network adjacent to (but independent from) the lipid membrane. Our data also indicate that the C-terminal domain of M1 is not needed for the establishment of protein-protein interactions and membrane deformation. Finally, we performed experiments at acidic pH (pH=5). In these conditions, M1 irreversibly loses its ability to multimerize. Our data indicate that M1 dimers can bind to GUVs but are not able to induce curvature, thus confirming that M1 multimerization is the molecular mechanism responsible for membrane deformation.

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## T-58. DNA-encircled lipid bilayer: a novel nano-scaled membrane-mimetic system

Karim Fahmy[1], Katarina Iric[1,2], Madhumalar Subramaniam[1,2], Jana Oertel[1], Nayan P. Agarwal[2], Michael Matthies[2], Xavier Periole[3], Thomas P. Sakmar[4], Thomas Huber[4], Thorsten-Lars Schmidt[2]

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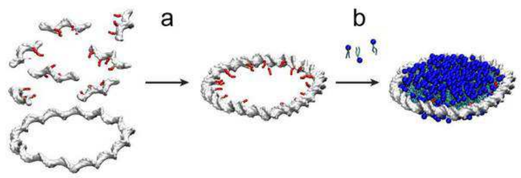
Lipid bilayers and lipid-associated proteins play a crucial role in biology. Since studies and manipulation in vivo are inherently challenging, several in vitro membrane-mimetic systems have been developed to enable the study of lipidic phases, lipid-protein interactions and membrane protein function. Controlling the size and shape or introducing functional elements in a programmable way is, however, difficult to achieve with common systems based on polymers, peptides or membrane scaffolding proteins. In this work we describe a route leveraging the unique programmability of DNA nanotechnology to create DNA-encircled bilayers (DEBs) as a novel nano-scaled membrane-mimetic. For this, alkylated oligonucleotides are hybridized to a single-stranded minicircle (ssMC) such that all alkyl chains point to the inside stabilizing the lipid bilayer. Atomic force microscopy (AFM), transmission electron microscopy (TEM) and coarse grain molecular dynamics (CGMD) simulations confirm the formation of discoidal lipid bilayer structures. Fluorescence spectroscopy was used to monitor lipid phase transitions and revealed head group-dependent lipid-DNA interactions at the bilayer rim. The DEB technology described herein provides unprecedented control of size, shape, stability and functionalization of engineered membrane nanoparticles and will become a valuable tool for biophysical investigation of lipid phases and lipid-associated proteins and complexes.




Assembly scheme of a DEB: a. ssDNA MCs (bottom) react with 7 complementary strands, each carrying 4 alkyl groups in red (top) forming a double-stranded DNA MC b. in the presence of lipids, a bilayer is formed inside the ds DNA MC, resulting in DEB formation.

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## Poster Presentations

### P-001. Nuclear inelastic scattering for identification of iron ligand modes in dinitrosyl iron complexes and nitrogenase single crystals

Hendrik Auerbach[1], Christina S. Müller[1], Christine Schiewer[2], Christian Trncik[3], Juliusz A. Wolny[1], Lena Scherthan[1], Andreas Omlor[1], Sakshath Sadashivaiah[1], Larissa Heimann[1], Ilya Sergeev[4], Olaf Leupold[4], Hans-Christian Wille[4], Oliver Einsle[3], Franc Meyer[2], Volker Schünemann[1]

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[2]Universität Göttingen, Department of Chemistry, Göttingen, Germany
[3]Universität Freiburg, Department of Biochemistry, Freiburg, Germany
[4]DESY, Hamburg, Germany

Vibrational iron ligand modes of $^{57}$Fe labelled complexes can be accessed via synchrotron based nuclear inelastic scattering (NIS). First results of NIS experiments performed in October 2017 at PETRA III, DESY are presented and compared to simulations of the experimental partial density of vibrational states (pDOS). The influence of protonation on the vibrational properties of a dinitrosyl iron complex (DNIC) has been investigated. DNICs are a product of the reaction of the messenger molecule NO with iron sulfur clusters and their reactivity might depend on the protonation state. The density functional theory based simulations confirm the experimentally observed distinct differences between the two protonation states and reproduce the intense features in the pDOS energy region > 500 $cm^{-1}$ typical for Fe-NO vibrations [1]. Moreover orientation dependent NIS experiments on CO-free and CO-inhibited $^{57}$Fe enriched nitrogenase single crystals have been performed. Despite the low signal to noise ratio, slight but significant orientation dependent differences are observed for low energy modes < 100 $cm^{-1}$ as well as in the region of characteristic Fe-CO modes above 460 $cm^{-1}$ [2].

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### P-002. Role of local membrane protein hydration for the stability of the $P_{IB}$-type ATPase CopA from Legionella pneumophila

Lisa Nucke[1], Jana Oertel[1], Karim Fahmy[1]
[1]Helmholtz-Zentrum Dresden-Rossendorf, Biophysics, Dresden, Germany

The active transport of ions across biological membranes is crucial for the homeostasis of the cell. One important trace element which needs to be tightly controlled is copper. Perturbations in the regulatory mechanisms can lead to severe diseases in humans such as Wilson"s disease which is caused by mutations in the copper-transporting ATPases ATP7A and ATP7B.[1] To completely understand the impact of the mutations, the copper transport mechanism needs to be understood on the molecular level. One crucial element seems to be the interaction of the ion hydration shell with the membrane protein interior which has recently been investigated for the $P_{IB}$-type ATPase CopA from Legionella pneumophila[2], a homolog to ATP7A and ATP7B.

Here, the role of hydration sites around the conserved copper-binding cysteine-proline-cysteine (CPC) motif for the stability of CopA was analyzed. Using two single-cysteine CopA mutants the site-specifically labeling of internal cysteines with a polarity-sensitive fluorophore could be achieved. The local fluorescence signal was combined with the circular dichroism signal of the α-helical signature region to correlate the protein unfolding event with the hydration state at the ion-binding site. Red shifts of the fluorescence emission maximum in both mutants indicate an increase in polarity in the vicinity of the CPC-motif with increasing temperature. Remarkably, above 40°C different unfolding pathways for the cysteine residues were detected. The data show that the two ion binding cysteines are structurally coupled to different protein domains, which is possibly caused by the helix breaking properties of the conserved proline residue positioned between the metal coordination sites.

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### P-003. How long-ranged are collective motions between biomolecules?

Christopher Päslack[1], Lars Schäfer[1], Matthias Heyden[2]

[1]Ruhr-University Bochum, Center for Theoretical Chemistry , Bochum, Germany
[2]Arizona State University, School of Molecular Sciences, Tempe, AZ, United States

We characterize correlated vibrational motions between a peripheral membrane protein and a lipid bilayer using molecular dynamics simulations. The collective motions are analyzed via correlations of atomic velocity fluctuations and further characterized as longitudinal currents similar to propagating acoustic modes. Interestingly, correlated vibrations, which are mediated by the solvation water, persist up to separation distances of 25 Å between protein and membrane surfaces. As this is more than twice the average distance between biomolecular surfaces in the crowded cytosol, this finding could have implications for biomolecular recognition and binding under physiological conditions.

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### P-004. Antigen processing at the atomic level: MD simulations of MHC-I and its peptide-loading complex

Olivier Fisette[1], Lars Schäfer[1]

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Antigens exposed at the cell surface by major histocompatibility complex class I (MHC-I) proteins enable self/non-self recognition by cytotoxic T cells, protecting the organism against viral infections and cancer-causing mutations. To perform their role, MHC-I must first be loaded with an antigenic peptide in the peptide-loading complex (PLC), a large multi-protein assembly whose structure and dynamics are still poorly understood.

Using all-atom molecular dynamics (MD) simulations in explicit solvent, we studied key elements of the PLC, how they stabilise MHC-I and catalyse antigen selection, and how they assemble to form the PLC. By combining these simulation results with a 6-Å resolution cryo-EM structure of the PLC, we have built an atomistic model of the complete complex.

We have shown how tapasin, a central component of the PLC, acts as both an MHC-I chaperone and a catalyst that accelerates the off-rate of low-affinity peptides to facilitate antigen triage. We have also shown how tapasin recruits the transporter associated with antigen processing (TAP) into the PLC via transmembrane interactions. Finally, by truncating antigens or removing them from the MHC-I binding groove, we made a spatially resolved map of MHC-I plasticity, revealing how peptide loading status affects key structural regions contacting tapasin.

Taken together, our MD simulations explain experimental kinetics and mutagenesis data, and represent the first in-depth, atomic-level study of the mechanism underlying the PLC, an important step towards a better understanding of adaptive immunity.

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### P-005. Structural Biology of Functional Amyloids forming Biofilms

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Many bacteria form biofilms to survive in stress-inducing conditions to achieve communal living. Biofilm-associated pathogenic microbes are protected from antimicrobial agents and host immune system attacks, as a result they are more infectious and difficult to treat. 80% of all human infections are related to bacterial biofilms. Proteins in the form of amyloid fibrils play a key role in maintaining the structural integrity of biofilms, and thus bolster resistance to antimicrobial agents. When these proteins are mutated, the biofilms are disrupted and bacteria become accessible to antibiotic treatments, a step which will be utilized for drug development. However, very little structural information exists about biofilms and their fibrillary components. Determining unknown structures and unraveling the structure-activity relationship of such fibrils will help to develop better therapies against antibioticresistant infectious bacteria in biofilms and amyloid formation in general. This forms the rational of my motivation. Here I will discuss our recent results from two different bacteria and discuss findings in general, bu using mainly solid-state NMR but along with other high resolution techniques.

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### P-006. A novel setup for time-resolved IR spectroscopy on Cytochrome c Oxidase

Pit Langner[1], Federico Baserga[1], Hendrik Mohrmann[1], Joachim Heberle[1]

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Cytochrome c Oxidase (CcO) is the fourth and terminal complex in the mitochondrial respiratory chain. Physiologically, it uses four electrons provided by cytochrome c to pump four protons into the intermembrane space, while catalytically reducing oxygen to water. The atomic details of the sequential steps that go along with this redox-driven proton translocation are still a matter of debate. We use CcO from Rhodobacter sphaeroides to study the correlation between the active center's redox state and the protonation events in the enzyme's catalytic cycle. Time-resolved infrared spectroscopy is a well-established method to investigate transient protonation changes but applying it to CcO poses a plethora of challenges. Our goal is to use a quantum cascade laser (QCL) setup and slow-flowing, CO poised CcO solution in a microfluidics channel transparent to mid-infrared radiation to eventually study oxygen binding on the reduced enzyme after CO flash-off. We report on a novel setup designed for this purpose.

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### P-007. Pore-spanning membranes: a tool to study single vesicle content release in SNARE-mediated membrane fusion

Peter Mühlenbrock[1], Claudia Steinem[1]

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The transmission of electrical signals between neurons in the nervous system is a fast and highly regulated process in which fusion of curved synaptic vesicles with the planar presynaptic membrane leads to neurotransmitter release into the synaptic cleft. Membrane fusion in neuronal signal transmission is driven by so-called SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) proteins.

To understand the mechanisms of this process a unique in vitro fusion assay has been established that enables the detection of individual fusion events. Pore-spanning membranes (PSM) on closed cavities serve as a model system that mimics the planar geometry and the tension of the presynaptic membrane. SNARE-mediated fusion of curved unilamellar vesicles with the target membrane allows to monitor the release of vesicular content into the opposite aqueous compartment. Spinning disc confocal microscopy (SDCM) is used to record the whole process from vesicle-docking to content release with high time resolution in a sensitivity of single fusion events.

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## Poster Presentations

### P-008. Optimizing crystal size of photosystem II by macroseeding: toward neutron protein crystallography

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Photosystem II (PSII) catalyzes the photo-oxidation of water to molecular oxygen and protons. The water splitting reaction occurs inside the oxygen-evolving complex (OEC) via a $Mn_4CaO_5$ cluster. To elucidate the reaction mechanism, detailed structural information for each intermediate state of the OEC is required. Despite the current high-resolution crystal structure of PSII at 1.85 Å and other efforts to follow the structural changes of the $Mn_4CaO_5$ cluster using X-ray free electron laser (XFEL) crystallography in addition to spectroscopic methods, many details about the reaction mechanism and conformational changes in the catalytic site during water oxidation still remain elusive. In this study, we present a rarely found successful application of the conventional macroseeding method to a large membrane protein like the dimeric PSII core complex (dPSIIcc). Combining microseeding with macroseeding crystallization techniques allowed us to reproducibly grow large dPSIIcc crystals with a size of ~3 mm. These large crystals will help improve the data collected from spectroscopic methods like polarized extended X-ray absorption fine structure (EXAFS) and single crystal electron paramagnetic resonance (EPR) techniques and are a prerequisite for determining a three-dimensional structure using neutron diffraction.

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### P-009. Investigations on microbial channelrhodopsins studied by time-resolved FT-IR an UV/Vis spectroscopy

Franziska Pranga-Sellnau[1], Mattia Saita[1], Ramona Schlesinger[1], Joachim Heberle[1], Victor Lórenz-Fonfría[2,3]

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The discovery of light-gated ion channels opened up the field of optogenetics, the combination of optical and genetic tools in order to control single cells. Two of the best-studied ChRs are CrChR2 from Chlamydomonas reinhardtii and CaChR1 from Chlamydomonas augustae. In CrChR2 first results on a molecular level were accomplished. However, there are two photocycle models derived from experiments performed under multiple turnover conditions (electrophysiology) and single turnover conditions (spectroscopy). We could show that the long-lived P4480-intermediate, which accumulates under multiple turnover conditions, is photosensitive and its photoexcitation leads into a secondary photocycle. Our results are capable of combing the two photocycle models.
Whereas advanced results have been achieved for CrChR2, we know less about CaChR1. I performed time-resolved FT-IR step scan experiments to provide time-resolved molecular insides into the protein over a broad spectral range.

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### P-010. Investigating proton transfer with SERRS and electrophilic addition of isocyanates in the catalytic centre of cytochrome c oxidase

Fabian Kruse[1], Inez Weidinger[1]
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Within this work surface enhanced resonance Raman spectroscopy (SERRS) is combined with an electrochemical setup to investigate proton transfer in cytochrome c oxidase (CcO). In this work the protein in its resting state, the fully oxidized “as-isolated” form without any further electron injections, was investigated. While under turnover conditions, each time an electron is donated to the enzyme, a proton is pumped across the cell membrane, presumably triggered by the reduction of heme a, no proton-pumping is observed even though six electron equivalents are donated to bring the protein from its resting state back to the fully reduced state, thus in the presence of oxygen and further electron equivalents back to turn-over-conditions. In the resting state a peroxide bridge is thought to be presence which could suppress the proton pump. By using an excitation-laser with a wavelength of 647nm, which is close to a ligand to metal charge transfer at 655nm visible in UV-VIS, we investigate a Raman vibration at 750cm-1, which could originate in the stretch vibration of a bridging peroxide. Furthermore, advantage is taken of the fact that CcO ends up in a mixed valence state, with heme a being reduced, after the catalytic center is inhibited, when the protein is immersed in aqueous solutions with high concentrations of KCNO. The benefit of this new procedure is that the mixed-valence state is achieved, without any electrochemical treatment, or without the use of a reducing agent, where the electron-donation pathway remains unclear. Furthermore, we used the site-specificity of H/D sensitive propionate vibrational modes by using them as markers for proton accessibility to the two active heme centers.

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### P-011. Electrostatic pKa calculations of the tetrapyrrole chromophore in phytochromes

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Phytochromes are biological red-light photoreceptor and can be found in bacteria and plants. All phytochromes utilize a covalently attached tetrapyrrole chromophore (TPC) that enables photoconversion between red-absorbing (Pr) and far-red-absorbing (Pfr) states. An accurate characterization of the protonation state of the TPC and all titratable residues in the chromophore binding pocket (CBP) is essential for understanding the signal transduction mechanism at atomic resolution. In this work, we applied the electrostatic approach based on the solution of the linearized Poisson Boltzmann (LPB) equation, implemented in the Karlsberg2+ software, to two phytochromes species. The TPC was divided into three fragments: the propionic side chain on ring C (psC), the propionic side chain on ring B (psB) and the chromophore core. The titratable psB and psC fragments were treated in the same way as the carboxylic group of an aspartic acid. In order to treat the TPC as a titratable site, atomic partial charges of the TPC were generated with the quantum chemical program Jaguar v.7.7 using the B3LYP DFT functional and 6-31G** basis set. The TPC geometry was optimized quantum chemically and then, the electrostatic potential of the TPC was computed based on the electronic wave function and charges of the nuclei using the same method as for geometry optimization. Atomic partial charges of the TPC were generated based on this electrostatic potential, using a two-stage restraint-electrostatic-potential(RESP) procedure. Finally, pKa values were computed by combining electrostatic energy calculations and MD simulations using Karlsberg2+. Conclusively, we present here a new methodology to determine the protonation state of the TPC and all titratable residues in the chromophore binding pocket.

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### P-012. Salt-induced assembly of fibrinogen into nanofibrous scaffolds

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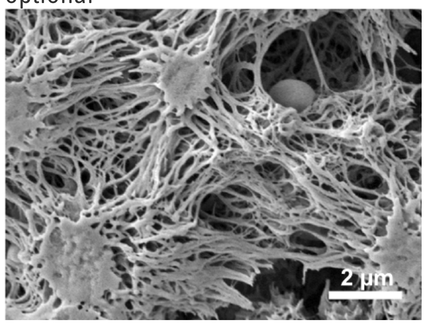
The plasma protein fibrinogen (Fg) plays a pivotal role in blood clotting and wound healing. During the coagulation cascade Fg is cleaved by the enzyme thrombin. Fibrinopeptides A and B are released and Fg is converted to nanofibrous fibrin network. In previous studies Fg was assembled into nanofibers in vitro using acidic buffers, electrospinning or hydrophobic surfaces. Here, we present a novel in vitro concept to induce fibrillogenesis of Fg without the influence of thrombin.

Aqueous Fg solutions were deposited onto various substrate materials. Subsequently, different salt buffers were added and the samples were dried to facilitate scanning electron microscopy analysis. With protein concentrations of at least 2 mg/ml we observed the formation of dense nanofiber assemblies on gold, hydrophilic glass and glass with an amino silane modification (Fig. 1). We found that a pH range of 7 to 9 facilitated fibrillogenesis of Fg. Phosphate buffered saline and other Na- or K-based buffers induced fiber formation with diameters of 100 to 200 nm. By increasing the salt concentration we could assemble Fg into nanofibrous scaffolds, which were several centimeters large and several micrometers thick.

When a thrombin-inhibitor was added to the Fg solution prior to self assembly we also observed fiber formation. Interestingly, self-assembled Fg nanofibers were found to dissolve again upon re-hydration. Thus, we assume that no cleavage of the fibrinopeptides A and B has occurred during the salt-induced self assembly, as this molecular change would have resulted in an insoluble fibrin network. Moreover, since Fg assembled into fibers on substrates with different surface chemistries we suppose that the main driving force underlying this fibrillogenesis mechanism are salts in combination with drying. In future studies we want to exploit this novel in vitro model system for Fg fibrillogenesis as versatile platform in synthetic biology to study wound healing mechanisms.

optional

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### P-013. Lipid vesicles containing cholesterol catalyse Aβ42 aggregation through a heterogeneous nucleation pathway

Céline Galvagnion[1], Johnny Habchi[2], Sean Chia[2], Thomas CT Michaels[2], Mathias MJ Bellaiche[2], Francesco S Ruggeri[2], Michele Sanguanini[2], Ilaria Idini[3], Janet R Kumita[2], Emma Sparr[4], Sara Linse[3], Christopher M Dobson[2], Tuomas PJ Knowles[2], Michele Vendruscolo[2]

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Alzheimer's disease is a neurodegenerative disorder associated with the aberrant aggregation of the amyloid-β peptide. Although increasing evidence implicates cholesterol in the pathogenesis of Alzheimer's disease, the detailed mechanistic link between this lipid molecule and the disease process remains to be fully established. To address this problem, we adopt a kinetics-based strategy that reveals a specific catalytic role of cholesterol in the aggregation of Aβ42 (the 42-residue form of the amyloid-β peptide). More specifically, we demonstrate that lipid membranes containing cholesterol promote Aβ42 aggregation by enhancing its primary nucleation rate by up to 20-fold through a heterogeneous nucleation pathway. We further show that this process occurs as a result of cooperativity in the interaction of multiple cholesterol molecules with Aβ42. These results identify a specific microscopic pathway by which cholesterol dramatically enhances the onset of Aβ42 aggregation, thereby helping rationalize the link between Alzheimer's disease and the impairment of cholesterol homeostasis.

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### P-014. Insights into the secondary nucleation of alpha-synuclein

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Alpha-synuclein (aSyn) is a natively unfolded protein predominantly localized in the presynaptic terminals of neurons where it most likely regulates the release of synaptic vesicles [1,2]. It has been shown that aSyn fibrils are the major component of abnormal neuronal aggregates known as Lewy bodies, the characteristic hallmark of Parkinson's disease [3]. Amyloid fibrils arise through primary nucleation from soluble monomers, which in the case of aSyn requires suitable surfaces with an affinity for the proteins [4]. The fibrils can also elongate through the addition of monomers onto their ends which, acting as template, induce the freshly added monomers to adopt the conformation of the seeds [5]. Secondary nucleation, on the other hand, involves the binding of monomers onto the surface of the pre-existing fibrils, leading to the formation of new fibrils. It is still unclear whether the fibrils formed through secondary nucleation adopt the morphology of the seeds, as in the case of fibril growth. The existence of such a secondary nucleation pathway has been already described for the amyloid-β (Aβ) peptide Aβ42 [6] and as well for aSyn [7]. Here we have investigated whether the secondary nucleation of aSyn also transmits the properties of the seed fibrils.

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## Poster Presentations

### P-015. Conformational and protonation dynamics at the surface of phytochromes

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Phytochromes are red-light photoreceptor proteins that regulate a variety of responses and cellular processes in plants, bacteria, and fungi. They utilize light to control various biological processes and share similar molecular processes, which include light absorption by the chromophore, transient protonation /deprotonation and protein structural changes, eventually leading to activation and signal transduction. Phytochromes act as photochemical switches, which interconvert between a red (Pr) and a far-red (Pfr) absorbing state [1]. In this work Cph1 from Synechocystis and Agp1 from Agrobacterium tumefactions are investigated.

We utilize time-resolved fluorescence measurements to study the conformational dynamics of phytochrome. Fluorescence probes are attached to the protein by cysteine labeling and were used to investigate the fluorescence depolarization (fluorescence anisotropy) as a function of pH. Changes in final anisotropy as a function of pH identified conformational changes in the protein that seem to correlate with chromophore deprotonation.

Furthermore, the surface attached pH-sensitive probes were used to study the bulk and surface specific protonation states. Titration experiments of the labeled protein show differences in the protonation behavior between the pH-probe at the protein surface and in solution. To reveal the intermediate states during Pr to Pfr transition, the kinetic and protonation signal was measured by time-resolved absorption spectroscopy.

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### P-016. Human Argonaute 2 in action: Mechanistic insights into the key player of RNA interference by SLAM-FRET

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Human Argonaute 2 (hAgo2) is the key player of RNA interference (RNAi), a posttranscriptional mechanism that regulates a major portion of human genes, including a high number of genes involved in disease-relevant processes. To exert its function, hAgo2 binds short RNAs that guide the enzyme to its cognate mRNA target via base complementarity. Based on the degree of complementarity, the target mRNA is either bound and translation of the mRNA is inhibited accompanied by the recruitment of proteins that lead to degradation of the mRNA. Alternatively direct cleavage of the bound mRNA by hAgo2 is possible. As common to transient protein-nucleic acid complexes, interactions have to be established and disrupted to allow substrate exchange. This demands structural flexibility in proteins like hAgo2. X-ray crystal structures provide valuable information about stable conformations that hAgo2 adopts upon guide and target binding. However, the dynamic aspect of hAgo2 action could not be elucidated so far as hAgo2 is not accessible to common protein labelling schemes for SmFRET measurements. In order to solve this problem and to use the native state of hAgo2 including all necessary post-translational modifications like e.g. phosphorylation, we developed the SLAM-FRET (**S**ite-specific **la**belling of endogenous **m**ammalian proteins for single-molecule FRET measurements) workflow (1). Making use of this method, we conducted smFRET measurements with fluorescently labelled hAgo2 protein and/or labelled guide and target RNAs. This way, we observed the conformational evolution of hAgo2 throughout its activity cycle. Among others, we found that the phosphorylation state of hAgo2 influences the conformational space and that guide-loaded hAgo2 adopts two conformational states not observed in crystal structures. Hence, our data complement the structural information on hAgo2 and provide insights into the dynamic interaction of RNAs with hAgo2.

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### P-017. Cell free expression to investigate site-specific dynamics of GPCR

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G protein-coupled receptors (GPCRs) are known to be highly dynamic proteins and the dynamics are thought to be an important aspect of their function as signaling molecules. Recently, we were able to get insight into the dynamics of peptide-binding class A GPCRs by solid-state NMR spectroscopy. It was possible to characterize the dynamics of the receptors in the presence and in the absence of different ligands and embedded in different membranes [1,2,3]. However, the order parameters that were derived from the Dipshift experiments for the fully 13C labeled neuropeptide Y and GHS receptors represent average values of all residues and did not resolve any specific properties of distinct regions, e.g. the loop or the transmembrane regions. Here, we present a cell free expression system for the production of GPCRs in the precipitated form. Besides an accelerated production, easier purification of the expressed protein and no toxicity concerns, this system offers the great potential of selective isotope labelling without being hampered by the metabolism of a living host environment. The resulting NMR spectra show a reduced spectral complexity and allow the investigation of specific amino acid sites, e.g. with respect to receptor activation.

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### P-018. Force-dependency of Cas9 target recognition investigated with a DNA origami-based nanoscopic force clamp

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Among the many clustered regularly interspaced short palindromic repeats (CRISPR) system variants, the type II CRISPR system is widely used for gene editing applications. It contains a single effector protein, the endonuclease Cas9 (CRISPR associated), which associates with an RNA duplex consisting of a CRISPR RNA (crRNA) and a trans-acting CRISPR RNA (tracrRNA) to specifically cleave double-stranded DNA (dsDNA) targets complementary to the crRNA. Additional to sequence complementarity, a protospacer adjacent motif (PAM) is required for efficient target recognition and cleavage. For editing purposes, sgRNA-associated Cas9 needs to be efficiently imported into the nucleus to encounter its target sequence. However, genomic DNA is seldom easily accessible, but often tightly packed within chromatin regions. The wrapping and bending of DNA induces tensions on the dsDNA and this might constitute a decisive factor for Cas9 efficiency and accuracy. We employed our recently developed DNA origami-based nanoscopic force clamp[1] to analyze whether the extent of strain on DNA influences Cas9 binding. This approach allows high throughput force measurements in the biological relevant piconewton range on the single-molecule level. Thereby, we exerted a defined force on the target DNA and monitored Cas9 binding on the single-molecule level using a fluorescence resonance energy transfer (FRET) signal between the donor-labeled target DNA and the acceptor-labeled Cas9-bound crRNA as readout. We can show that the binding behavior of the Cas9-sgRNA to its target DNA changes in a force-dependent manner and that high forces abolish Cas9-sgRNA binding to its target DNA. These results suggest that Cas9 is a force-sensitive enzyme and that strained DNA regions in the genomic DNA might be less efficiently recognized by Cas9.

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### P-019. Deciphering the functional composition of fusogenic liposomes


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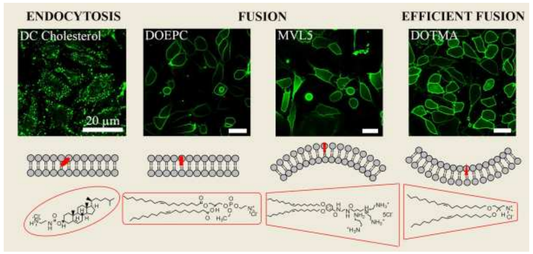
Current drug delivery strategies use the endocytic pathway to introduce biomolecules like proteins, DNA, or antibiotics into living cells. The main drawback of endocytic uptake is the degradation of the cargo. Compared to this, a more promising alternative for efficient molecular delivery is the induction of membrane fusion between liposomes and mammalian cells. Therefore special liposomes with extraordinary high fusion efficiency have been developed in our institute for such purposes. Due to a complete membrane mixing of the liposomal membrane and the cellular plasma membrane the cargo molecules can be effectively released into the cell cytoplasm avoiding its degradation. In the last decade their applications became more and more relevant, however the exact fusion mechanism is still to be elucidated.

Therefore the aim of this work has been to investigate those liposomes and their fusogenity with living mammalian cells dependent on lipid composition as well as environmental conditions. For liposomal characterization dynamic light and neutron scattering and calorimetry were applied. Fusion efficiency was investigated using fluorescence microscopy and spectroscopy, and flow cytometry. Our first results show that FLs need cationic lipids with inverted conical molecular shapes and aromatic components at a distinct concentration for the best fusion. While a neutral lipid is not mandatory, it can be used to control fusion efficiency. Neutral lipids with long and unsaturated chains and a small head group do not change liposomal fusion ability while those with saturated short chains and a big head group do, and in most extreme cases revert the uptake mechanism back to endocytosis. The thermotropic phase behaviours of endocytic and fusogenic liposomes by neutron scattering and freeze-fracture techniques.These results are under further investigation leading to better understanding of the fusion mechanism.

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### P-020. Mechanism of the intrinsic arginine finger in heterotrimeric G proteins

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Heterotrimeric G-Proteins mediate a plethora of physiological processes such as vision, scent and blood pressure regulation. The signal is encoded into surface alterations of the Gα subunit that binds GDP in its inactive state and GTP in its active state. The off-switch is achieved via GTP hydrolysis within the active site of Gα. When this process is hindered, e.g. by mutation of a residue called the intrinsic “arginine finger”, severe diseases occur. GTP hydrolysis is aided by a GTPase activating protein (GAP), for example RGS4 in the case of $G\alpha_{i1}$. The structure of both proteins was previously elucidated via Xray crystallography, however the GAP-mechanism of RGS4 upon $G\alpha_{i1}$ was still limited to a model of allosteric stabilization of the binding pocket.

We applied fourier transformed infrared (FTIR) spectroscopy using photocaged GTP to investigate the intrinsicand RGS4-catalyzed GTP hydrolysis reaction in $G\alpha_{i1}$ and extended the understanding of RGS catalysis toward a high-resolved model with sub-angstrom spatial and millisecond temporal resolution.The structural information from the FTIR experiments was decoded using hybrid QM/MM calculations.

Our results show that the intrinsic arginine finger in heterotrimeric G-proteins is bound to γ-GTP for intrinsic Gα, which was not visible in crystal structures due to the applied GTP analogs. Binding of RGS4 pushes the arginine finger from a monodentate γ-GTP coordination toward a bidentate α- γ-GTP coordination. This movement shifts charges within the substrate and induces a structural strain: the γ-GTP group becomes more planar and the α-GTP group is twisted to an eclipsed conformation. Altogether these electronic and structural alterations bring the substrate closer to the transition state of GTP hydrolysis and accelerate the reaction by two orders of magnitude.

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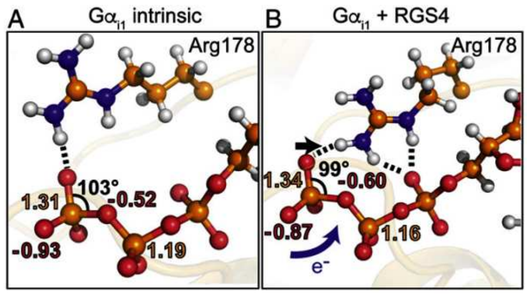


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### P-021. NMR studies to unravel the binding of the orphan ligand CXCL14 towards Glycosaminoglycans

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CXCL14, a soluble 9.4 kDa protein, is a novel, highly conserved chemokine with unprecedented features. Despite exhibiting the typical chemokine fold, its flexible N-terminus consists of only two amino acids; a domain that is usually attributed to chemokine receptor activation. This is intriguing, because CXCL14 is still an orphan ligand. A growing body of evidence points towards a multitude of immunomodulatory activities such as homeostatic immune surveillance and elimination of early neoplasmic transformations in skin and mucosae. Its strong anti-angiogenic properties may be a target for cancer therapy.

In the present study, we investigated the interaction of CXCL14 with different Glycosaminoglycans (GAGs) to map out GAG-specific amino acid signatures in order to understand the molecular level of GAG-binding. Therefore, we cloned, expressed and refolded CXCL14 with final yields of up to 4 mg highly pure protein per liter E. coli batch culture. The observable chemical shift perturbation in $^{1}H$-$^{15}N$-HSQC NMR spectra upon titration with different hexameric GAGs (dp6) suggests different primary binding sites. We hypothesize varying sulfation pattern of the GAGs confer specificity beyond simple electrostatic interactions, which is also supported by complementary computational methods.

Interestingly, the differences in the amino acid pattern are less pronounced, if longer GAGs (dp10) are investigated. Furthermore, titration experiments of Heparin dp10 were not feasible due to an immense loss in NMR signal intensity. However, upon heparin dp10 titration the solution remains clear and no line broadening in the spectra was observed. This indicates a tendency of CXCL14 to form stable, fast relaxing, high molecular weight oligomers in the presence of heparin, which were also shown in a preliminary CXCL14 crosslinking experiment in the presence of heparin.

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### P-022. Influence of the splicing variants ΔExon3 and ΔExon5 on α-Synuclein aggregation

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The aggregation of α-synuclein (αS) plays a key role in Parkinson"s disease (PD) and is also involved in other neurodegenerative diseases like dementia with Lewy bodies (DLB), Parkinson"s disease with dementia (PDD) and multiple system atrophy (MSA). α-synuclein is an intrinsically disordered protein that consists of 140 amino acid (aa). The influence of disease mutants leading to an early onset of PD has been studied extensively while the role of the naturally occurring splicing variants ΔExon3 and ΔExon5 remains to be elucidated. ΔExon3 lacks 14 aa in the N-Terminal region that is known to bind to phospholipid membranes. ΔExon5 lacks 28 aa in the C-Terminal region which contains the ligand binding domain of αS. We use polarized ATR-FTIR spectroscopy and a solid supported lipid bilayer (SSLB) as a biomimetic membrane to monitor the aggregation kinetics of the αS-variants as well as the effect of aggregation on the membrane integrity. Using polarized light enables to determine the orientation of molecular groups during the aggregation process. We could identify different aggregation kinetics for both splicing variants. Furthermore, they show different interactions with the lipid membrane. Since membrane damage is assumed to induce deleterious effects in PD, ΔExon3 and ΔExon5 might function as regulating proteins preventing aggregation. Mixing experiments with the full-length protein and its splicing variants will give further insights into the exact role of ΔExon3 and ΔExon5 in αS aggregation and might also yield new insights into the pathology of PD.

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### P-023. Towards the structure of TRAP transporters with an integrative approach of crystallography and PELDOR

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The tripartite ATP-independent periplasmic (TRAP) transporters are secondary membrane-transporters, which are widespread in bacteria but absent in eukaryots (1). Until today, many different substrates for TRAP transporters were identified, illustrating the importance of these transporters for prokaryotic cells. The best studied TRAP transporters are specific for N-acetylneuraminic acid and play an essential role in infections of humane-pathogenic bacteria (2). While the structure and function of the substrate-binding protein has been well-characterized, the membrane domains are less well studied (3). There is currently no experimental information about their 3D-structure as well as about the dynamics and interaction between all three domains during the transport.

To study the TRAP transporter membrane domains with X-ray crystallography and pulsed electron-electron double resonance spectroscopy (PELDOR), large amounts of pure protein are needed. Here we present, how the expression and purification procedure of the membrane domains were optimized with different extraction methods like nanodiscs or micelles. For the co-crystallization of all three domains the formation of the tripartite complex was investigated to find optimal complex-forming conditions. We use PELDOR spectroscopy to analyse the dynamics of the membrane protein in solution and to study the interaction between the substrate-binding protein and the membrane domains. Due to the diamagnetic characteristics of the TRAP transporter, it was necessary to introduce spin labels via cysteine site-directed spin labelling (SDSL) into the proteins. We present how surface accessible native cysteines were identified and how the labelling procedure was optimised.

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### P-024. Characterization of quinoxalinedione antagonist binding to the glutamate receptor LBD using a fluorescence quenching assay

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate neurotransmission and neuromodulation in the central nervous system and are hence important drug targets. Receptor activation and desensitization is controlled by glutamate-binding to the extracellular ligand binding domains (LBDs), which form bilobed, clamshell-like structures that close around the ligand. Glutamate, as well as orthosteric agonists and antagonists, contain a negatively charged carboxylate group, which strongly interacts with a conserved arginine side chain located in the binding pocket. One exception are quinoxalinediones, a widely used class of AMPA and kainate receptor antagonists, which lack a carboxylate group, but which are likely to deprotonate upon amide-iminol tautomerisation.

Here we investigate the binding of quinoxalinedione antagonists that deprotonate in the physiological pH range to the GluA2 LBD using an in vitro fluorescence quenching assay. We heterologously expressed the GluA2 LBD in Escherichia coli and purified it using affinity chromatography. Next, we established a fluorescence binding assay, which is based on the quenching of the intrinsic Trp fluorescence by quinoxalinediones. Taking inner filter effects into account, this assay can be used to directly determine the binding constants of suitable antagonists, such as DNQX, and it can be applied to measure the affinity of other ligands by performing competition experiments. We find that, indeed, pH values, which favor the deprotonation of quinoxalinediones increase the binding affinity to the GluA2 LBD. This may have important consequences for using quinoxalinediones in physiological experiments and warrants further studies addressing the underlying amide-iminol tautomerisation and structure-function relationships in related pharmacological compounds.

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### P-025. Investigation on the dynamics of single domain transmembrane helices via various methods of solid state NMR

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Intramembrane proteases play an important role in the functionality of living cells and errors in these processes can lead to a variety of illnesses e.g. Alzheimer"s Disease (A.D.). Despite the known importance of these enzymes their recognition mechanism for substrates is still poorly understood. One Hypothesis for this is the recognition by differences in the molecular dynamics especially of the transmembrane domains (TMD). To study these molecular dynamics $A\beta_{26-55}$ polypeptides, labelled specifically with $^{13}C$,$^{15}N$ and $^{2}H$, were reconstituted in multilaminar vesicles with different lipid compositions. Afterwards we measured the strength of $^{1}H$-$^{13}C$ and $^{1}H$-$^{15}N$ dipolar couplings under magic angle spinning. To get a broader understanding of our results we repeated these experiments with GWALP23 model peptides. We also investigated the influence of the putative hinge at the $Gly_{37}Gly_{38}$ motif on the molecular dynamics by introducing different mutations at the $Gly_{38}$ position. This gives us insight on the role of the specific sequence and the membrane environment on the geometry and molecular dynamics of the transmembrane part of APP. At we also performed measurements of the dynamics on the second TMD of $LacY_{36-67}$ a substrate of the protease GlpG with and without it"s cleaving protease. Because of these studies, we found that the behaviour of the investigated polypeptides is strongly governed by orientation and whole-body motions inside the membrane. Furthermore, we found a considerable influence of mutations and lipid environment on these properties of the peptide. We validate these findings by comparing them with $^{2}H$-spectra we measured on the same samples.

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### P-026. The SAM domain of the murine protein SLY1 dimerizes through a novel SAM domain dimer interface

Laura Katharina Kukuk[1,2], Andrew Dingley[1,2], Joachim Granzin[1], Renu Batra-Safferling[1], Karen Hänel[1], Luitgard Nagel-Steger[2], Sandra Beer-Hammer[3], Dieter Willbold[1,2], Bernd Koenig[1,2]

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Sterile alpha motif (SAM) domains are interaction modules, which mediate complex formation of proteins with other proteins, lipids and RNA. A prominent feature of SAM domains is their ability to self-associate in a variety of homo- and heterotypic interactions. The murine signalling adapter protein “SH3 containing protein expressed in lymphocytes 1” (SLY1), which is involved in immune regulation, contains a SAM domain of unknown function. In this study, the SAM domain of SLY1 is shown to exist in a monomer-dimer equilibrium. The structure of the SAM domain dimer was solved by NMR spectroscopy and X-ray crystallography, and revealed that SLY1 SAM forms a symmetric homodimer through a novel dimer interface that consists primarily of the two long C-terminal helices of the domains packing against each other. The equilibrium dissociation constant of the SLY1 SAM dimerization is in the lower micromolar range, showing that this dimer is at least one order of magnitude more stable than previously characterized SAM domain homodimers. The five amino acid residues at the N-terminus of SLY1 SAM have a drastic effect on dimer stability.

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### P-027. Allosteric activation of GDP-bound ras isoforms by bisphenol derivative plasticisers

Raphael Stoll[1]

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The protein family of small GTPases controls cellular processes by acting as a binary switch between an active and an inactive state. The most prominent family members are H-Ras, N-Ras, and K-Ras isoforms, which are highly related and frequently mutated in cancer. Bisphenols are widespread in modern life because of their industrial application as plasticisers. Bisphenol A (BPA) is the best-known member and has gained significant scientific as well as public attention as an endocrine disrupting chemical, a fact that eventually led to its replacement. However, compounds used to replace BPA still contain the molecular scaffold of bisphenols. BPA, BPAF, BPB, BPE, BPF, and an amine-substituted BPAF-derivate all interact with all GDP-bound Ras-Isoforms through binding to a common site on these proteins. NMR-, $SOS^{cat}$-, and GDI- assay-based data revealed a new bisphenol-induced, allosterically activated GDP-bound Ras conformation that define these plasticisers as Ras allosteric agonists.

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### P-028. Probing conformational changes in the GluK2 ligand-binding domain using fluorescence spectroscopy

Felix Borowski[1], Andreas Reiner[1]
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Ionotropic glutamate receptors (iGluRs), a class of transmembrane proteins that form tetrameric ligand-gated ion channels activated by glutamate, are the key mediators of fast excitatory transmission between neurons in the vertebrate central nervous system. Agonist binding to the ligand-binding domains (LBDs) of iGluRs leads to rapid channel opening, which allows for an immediate cation flux resulting in membrane depolarization, and subsequent receptor desensitization. A key aspect for understanding how ligand binding and channel gating are coupled is to study the conformational changes induced by ligand binding.

In order to investigate ligand-induced conformational changes on the level of the individual LBDs we heterologously expressed the discontinuous LBD of the GluK2 receptor subtype in Escherichia coli and purified it by immobilized metal affinity chromatography. The spectroscopical properties of the LBD were recorded in the presence of known agonists and antagonists, which lead to a decrease in the intrinsic Trp/Tyr fluorescence pointing to environmental changes around these fluorophores, or to fluorescence quenching in the case of DNQX, a known iGluR antagonist. Denaturant-induced unfolding of the apo-LBD revealed the presence of two transitions, which might be differentially affected by ligand binding.

We currently establish the introduction of an unnatural amino acid, p-azidophenylalanine (pAzF), using amber stop codon suppression, which enables us to specifically label the LBD with extrinsic fluorophores by Cu(I)-catalyzed click chemistry. Covalently attached fluorophores with high sensitivity regarding environmental changes should allow us to proceed with time-resolved studies to get more insight into structural rearrangements of the isolated LBD at high temporal resolution.

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### P-029. Single-shot submicrosecond infrared spectroscopy on proteins with quantum cascade lasers

Jessica Laura Klocke[1], Markus Mangold[2], Pitt Allmendinger[2], Andreas Hugi[2], Markus Geiser[2], Pierre Jouy[3], Jérôme Faist[3], Tilman Kottke[1]

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[3]ETH Zürich, Institute for Quantum Electronics, Zürich, Switzerland

Time-resolved vibrational spectroscopy is a valuable tool to investigate processes in proteins initiated by a photoreaction. To our knowledge, all available methods require many repetitions to acquire a series of infrared spectra with high time resolution. Therefore, irreversible processes are challenging to study and require excessive amounts of sample and application of a flowcell.

Here, we present a setup based on quantum cascade laser (QCL) dual-frequency-combs with a spectral coverage of 55 $cm^{-1}$ in the fingerprint region at a native resolution of 0.3 $cm^{-1}$. We simultaneously recorded spectra and kinetics of the proton pump bacteriorhodopsin (BR), which are in good agreement with those from step-scan FTIR experiments. Due to the 320 ns time resolution of the setup, even a submicrosecond intermediate of the photocycle was characterized. In particular, the sequence of intermediates could even be resolved in a single-shot experiment. We extracted spectra of four intermediates as well as their kinetics at different pH values after a single excitation, showing the characteristic pH dependency of the BR photocycle.

In a second setup we combined a broadly tunable external-cavity QCL with a flowcell, to significantly extend the accessible spectral range and to achieve 30 ns time resolution in reaction kinetics over a broad time range with ns time resolution. In comparison to step-scan FTIR experiments the sample consumption can be drastically reduced by the possibility to focus the beam to the diffraction limit at much higher probe light intensity.

With these two complementary approaches we aim to pave the way for the analysis of irreversible reactions with high time resolution, broad spectral coverage and minimal sample consumption.

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### P-030. Analyzing molecular interactions of the tumor suppressor protein p53 with the biopolymer poly(ADP-ribose) by ATR-FTIR spectroscopy

Annika Krüger[1], Alexander Bürkle[1], Aswin Mangerich[1], Karin Hauser[1]
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The post-translational modification poly(ADP-ribosyl)ation (PARylation) is one of the first cellular signaling events upon DNA damage. It regulates specific DNA repair mechanisms, chromatin remodeling and gene transcription and therefore fulfils important functions during genome maintenance. In addition to covalent PARylation, PAR can interact with proteins in a non-covalent manner. Previous studies revealed that non-covalent PAR binding to proteins regulates macromolecular complex formation with significant consequences on protein functions. However, the mechanisms and functional consequences of these interactions are only partially understood. We developed a novel approach based on attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to obtain dynamic structural information of PAR-protein interactions under physiological conditions. The approach includes the functionalization of the crystal surface with a polyethylene glycol (PEG) layer to prevent unspecific protein adsorption and the specific immobilization of PAR or proteins, which than allows the direct study of the non-covalent interaction. The tumor suppressor protein p53 is in the focus of our investigations as it exhibits high affinity for PAR. Recently, we demonstrated that the non-covalent interaction is mainly mediated via the C-terminal domain of p53 with significant biochemical and cellular consequences. The C-terminal domain is intrinsically disordered and plays a central role in the regulation of p53 including its DNA binding ability. By analyzing different p53 variants with our ATR-FTIR spectroscopic approach, we show that PAR binding requires the C-terminal domain of p53 and furthermore that the presence of PAR can very efficiently lead to the release of p53 from DNA. Combined with our structural data our results provide molecular insights into the non-covalent binding mechanism of p53 and PAR.

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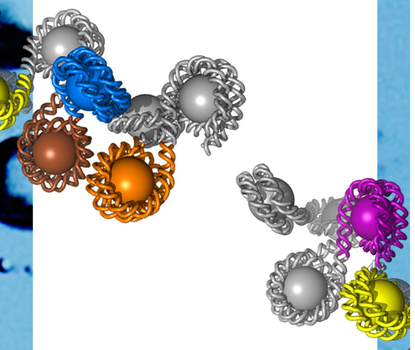



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### P-031. Late-endosomal SNAREs – Towards the characterization and establishment of a novel SNARE-family

Stefan Krüger[1], Jörn Dietz[2], Andreas Janshoff[2], Claudia Steinem[3]

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Protein-mediated membrane fusion is one of the essential processes throughout living cells. Due to their ubiquitous nature, these processes need to be highly regulated. One of these protein families are the so called SNARE (**S**oluble **N**SF-**a**ttachment **re**ceptor) proteins. Several SNARE-families have been identified in the last few decades, including the late-endosomal SNAREs. These proteins mediate the fusion processes between endosomes and lysosomes leading to a highly regulated mechanism in substrate sorting throughout living cells.

Due to the involvement of several fusion and chaperone proteins alongside with various lipids at the fusion site, the investigation of those processes demands for bottom-up solutions. In the last two decades, several of these systems have been established in literature. From vesicle-vesicle fusion set-ups in bulk-solution to various single event-based approaches a large variety of techniques have emerged. The vesicle-vesicle fusion assays allow for simple testing and quantification of protein activity by means of fluorescence spectroscopy via a lipid-mixing dequenching assay. More advanced set-ups like the colloidal probe assay allow for an analysis of kinetics and mechanics on a single event level.

We here present the data concerning the vesicle fusion assay in dependence of lipid composition as well as the influence of Ca2+-ions on the kinetics and fusion efficiency mediated by late-endosomal SNAREs.

Furthermore we show preliminary data obtained with the colloidal probe assay by means of AFM and confocal fluorescence microscopy. We particularly focus on the lateral mobility of the investigated bilayers by FRAP analysis.

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### P-032. Towards understanding the mechanism of decatenation by topoisomerase IV

Jana Hirsch[1], Dagmar Klostermeier[1]
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Topoisomerases play an important role in the cell as they regulate the topological state of the DNA during replication and transcription. They are divided into two families: type I topoisomerases cleave one strand of the DNA and change the linking number in steps of one; type II topoisomerases cleave a double strand and change the linking number in steps of two. Type IIA topoisomerases comprise DNA gyrase, eukaryotic topoisomerase II and topoisomerase IV (topo IV). These enzymes have a similar overall structure but different main tasks in the cell: gyrase is the only topoisomerase that can negatively supercoil DNA, topoisomerase II relaxes negative supercoils and topoisomerase IV decatenates freshly replicated DNA. All reactions are ATP-dependent. In the past a strand-passage mechanism for all the three reactions was proposed. An opening and closing of three protein-protein interfaces, so-called gates, leads to the passage of one DNA strand through the gap in another DNA strand. For this passage the opening and closing of one of these interfaces, the DNA-gate, is essential, however it could not be detected so far.

We investigate the mechanism of ATP-dependent decatenation by topo IV. Specifically, we want to observe the opening of the DNA-gate during strand passage and decatenation using single-molecule FRET. To achieve this goal, we have introduced cysteines at different positions at both sides of the DNA-gate and labeled these cysteines with donor and acceptor dyes. Single-molecule experiments on topo IV in solution show a high FRET efficiency in the absence of DNA, indicating that the DNA-gate is closed. Next, we will follow conformational changes of the DNA-gate during the decatenation reaction. These studies will help to understand the role of conformational changes that lead to decatenation, and might reveal differences in the mechanisms of different type IIA topoisomerases.

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### P-033. Structural identification of a novel interprotomer binding pocket in the capsid of enteroviruses preventing conformational change

James Alexander Geraets[1], Rana Abdelnabi[2], Yipeng Ma[2], Carmen Mirabelli[2], Justin Wayne Flatt[1], Ausra Domanska[1], Leen Delang[2], Dirk Jochmans[2], Venkatesan Jayaprakash[3], Barij Nayan Sinha[3], Pieter Leyssen[2], Johan Neyts[2], Sarah Jane Butcher[1]

[1]University of Helsinki, Institute of Biotechnology, University of Helsinki, Finland
[2]KU Leuven, Rega Institute for Medical Research, Leuven, Belgium
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Enteroviruses are responsible for a significant number of infectious diseases worldwide for which there are no prophylactic or therapeutic treatments available. Capsid binders such as pleconaril and vapendavir, the best-studied class of enterovirus inhibitors, bind in a hydrophobic pocket in protein VP1, expelling a lipid factor from the pocket and blocking virus attachment/uncoating. However these classical capsid binders failed clinical trials and additionally are inactive against some important pathogenic enteroviruses, such as rhinovirus C and parechovirus, as these lack the hydrophobic pocket. Here we found a novel druggable pocket on the Coxsackievirus B3 capsid surface, effectively targeted by a benzene sulfonamide derivative. We solved a 4.0 Å cryo-electron microscopy structure of the virus-inhibitor complex that unveiled an interprotamer binding pocket formed by three viral capsid proteins: two VP1 and one VP3. We show that the compound interferes with an early step in the virus replication cycle. Mechanistically we propose that the compound stabilizes a key region of the virion, preventing conformational expansion that activates the particle for RNA release. Here we present structural data and recent work on developing broader-spectrum enterovirus inhibitors that are opening a new avenue for enterovirus drug discovery.

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### P-034. Structural basis of inward rectification in $K^+$ channels

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Phycodnaviruses, which infect marine algae, code for potassium channels, like Kmpv1 and KmpvSP1( =$K^+$ channel from Micromonas pussila viruses). These proteins are with less than 100 amino acids very small but still exhibit the typical architecture of canonical $K^+$ channels. Functional expression of the two channels in cells and in planar lipid bilayers show that they exhibit a strong inward rectification, which resembles the mechanism of rectification in mammalian Kir channels. To uncover the structural basis for this rectification we compared the two channels with Kmpv1. This viral $K^+$ channel has a similar amino acid sequence but generates an ohmic conductance. A series of point mutations and chimeras of the respective channels reveal that the rectification is independent on the selectivity filter domain of the channels. Only a chimera, in which the initial part of the first transmembrane domain in KmpvSP1 is replaced by the equivalent domain of Kmpv1, looses the rectification property. Collectively these data underscore a mayor contribution of the outer transmembrane domain on the mechanisms of Kir type inward rectification.

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### P-035. ATR-IR-spectroscopy for conformational activity screening in drug discovery - a study on HSP90

Jörn Güldenhaupt[1], Marta Amaral[2,3], Carsten Kötting[1], Jonas Schartner[1], Djordje Musil[2], Matthias Frech[2], Klaus Gerwert[1]

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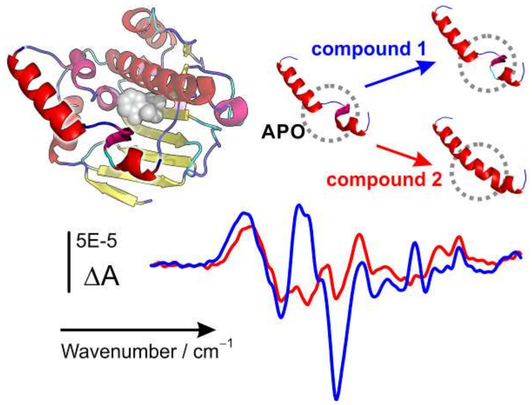
A detailed investigation of protein-ligand interactions is crucial in drug development. However, most available techniques lack either structural or temporal resolution. In contrast, attenuated total reflection Fourier-transform (ATR-FTIR) spectroscopy can monitor protein-ligand interactions label-free with temporal and chemical resolution. IR spectroscopy monitors the amide vibrations of the protein backbone and can thereby detect small secondary structure transitions (2 - 5% residues involved).

Here we immobilize the chaperone HSP90, an molecular target for drugs against several diseases including cancer, on an ATR-crystal. HSP90 was immobilized on germanium internal reflection elements (IREs) by a multistep surface modification which includes self-assembled monolayers of thiols and protein immobilization via His-tag. Ligand interaction of HSP90 measurements were performed with 19 inhibitors of two binding types. Inhibitors of one group induce a helix transition near the nucleotide binding niche (helix-binder), whereas the others bind without changing the protein conformation (loop-binder).

The ligand interaction was monitored time resolved within an automated flow-through system. The obtained timeseries of infrared spectra were analyzed with multivariate curve resolution alternating least square (MCR-ALS) fitting. From amide difference bands of the extracted component spectra we assigned all inhibitors correctly to their specific binding mode. From the extracted concentration profiles we obtained kobs values of the ligand disscociation which strongly correlate with reference koff values from surface plasmon resonance (SPR) measurements. The observed secondary structure changes and the respective kinetic values are in agreement with X-ray data and SPR experiments but the ATR-FTIR data is obtained in a few hours in a single experiment. This approach used in higher throughput could be utilized as screening technique for drug discovery in future.

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### P-036. Exploring the reductive phase of Cytochrome c Oxidase: assignment of hemes redox states and relative structure changes through potential-resolved FTIR

Federico Baserga[1], Hendrik Mohrmann[1], Sven T. Stripp[1], Joachim Heberle[1]
[1]Freie Universität Berlin, Physics, Berlin, Germany

Cytochrome c Oxidase (CcO) is a protein central to cellular respiration. Together with other enzymes, it contributes to the creation of the proton gradient essential to ATP synthesis. It is well known that CcO reduces molecular oxygen to water and pumps protons while undergoing a cycle initiated by electron injection from Cytochrome c. At the beginning of this catalytic cycle, the reduced Cytochrome c transfers electrons to the CuA cofactor of the enzyme. This step is followed, respectively, by the reduction of the metal centers of heme a and heme a3 and triggers a series of changes in the residues neighboring the active center. The mechanistic details of this machinery are not yet completely understood, partially because of the deficiency of time-resolved data providing structural information on the physiological cycle. We combine steady-state attenuated total reflection (ATR) FT-IR spectroscopy with electrochemistry in order to disentangle structural changes coupled to the reduction of single heme cofactors. We can control the redox state of their metal centers by mediated electron injection. After binding carbon monoxide to the active center of CcO and raising the applied potential, we monitor the shift of the C≡O stretching vibration, and ultimately its disappearance due to unbinding. CO is utilized both as a marker for the completely reduced state and as a probe for the Vibrational Stark Effect arising from the electric field of the reduced heme a on the oxidized heme a3. This approach allows us to clearly separate potentials corresponding to the oxidation states of the protein"s cofactors and to correlate them with the protein"s IR difference spectra.

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## Poster Presentations

### P-037. Structure of the bifunctional secretin PilQ from T. thermophilus


Edoardo D'Imprima[1], Ralf Salzer[2], Ramachandra Bhaskara[3], Janet Vonck[1], Gerhard Hummer[1,3], Werner Kühlbrandt[1], Beate Averhoff[1,2]

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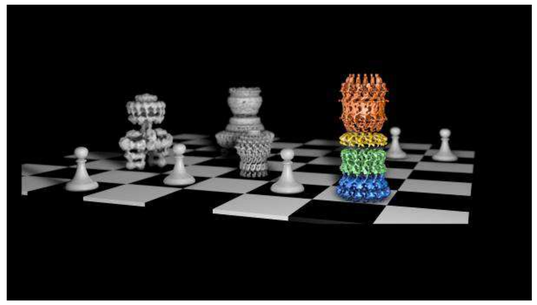
Natural transformation is one of the modes of horizontal gene transfer by allowing bacteria to take up free DNA from the environment. By natural transformation bacteria can adapt to human host and, for example, can spread the multi-drug resistance: one of the main public health threats. Competence proteins consists of multi-component transport machineries widely conserved through bacteria world. Among these nanomachines, members secretins protein family, highly conserved in Gram-negative bacteria, play a key role in natural transformation and often exhibit a dual role in both protein secretion/ biogenesis and DNA uptake. Here we present the first complete native structure of the secretin PilQ from the type 4 pilus system of Thermus thermophilus by cryo-electron microscopy.



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### P-038. A unifying photo-cycle model for Channelrhodopsin-2

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In optogenetics activatable cells like neurons are excited by light with high temporal and spatial resolution In order to allow such remote control of cells using light as a trigger microbial rhodopsins are used as optogenetic tools. Here we introduce a new photo-cycle model for Channelrhodopsin-2 (ChR2), which is a milestone for future optogenetic applications.

Microbial rhodopsins are light-gated cation/anion channels and ion-pumps. They are transmembrane proteins comprising seven transmembrane helices with retinal as a chromophore. ChR2 is widely used as an optogenetic tool. A detailed mechanistic understanding of these optogenetic tools is necessary to improve their properties depending on the respective application. Therefore, we used a combination of experimental (FTIR- and Raman-Spectroscopy and electrophysiological measurement) and theoretical (homology modeling, molecular dynamic (MD) simulation) techniques, to identify the molecular mechanism of ChR2.

With these methods we reveal an early splitting of the photo-cycle which makes a new photo-cycle model necessary for ChR2. By using FTIR- and Raman-Spectroscopy we were able to identify marker bands for a 13-cis,C=N-anti or a 13-cis,C=N-syn retinal conformation. We could deduce that 13-cis,C=N-syn represents a second closed-channel state. This state is identical to the long lived $P_{480}$-state, which has been previously assigned to a late intermediate. Additionally, by MD simulations we could describe the contact pattern in the early intermediate of both cycles. In combination with electrophysiological measurements we could observe high conductance in the open-channel state of the anti-cycle (including 13-cis,C=N-anti retinal) and slowly-decaying state with small conductance in the open-channel state of the syn-cycle (including 13-cis,C=N-anti retinal). These parallel anti- and syn-cycles provide a unifying photo-cycle model for ChR2, explaining the inactivation and ion selectivity of ChR2.

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### P-039. The number of SNARE complexes changing conformation during vesicle fusion

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The SNARE (Soluble NSF Attachment Receptor) complex is composed of the proteins synaptobrevin 2, syntaxin1 and SNAP25. The formation of SNARE complex is thought to provide the force and energy to overcome the energy barrier for membrane fusion. Extensive studies have provided estimates of the numbers of presynaptic proteins present at a release site without information on how many of them actually participate in fusion by exerting a conformational change. In our study, the SCORE2 (SNARE Complex REporter2), a FRET probe of SNAP25 was generated by inserting the FRET donor mCerulean3 and acceptor Venus at the N-termini of two SNAP25 SNARE motifs SN1 and SN2, respectively. The SCORE2 fully rescued the release capability in SNAP25-/- cells without affecting the frequency, size and kinetics of quantal release events. By combining the electrochemical detector (ECD) and TIRF-FRET imaging, we localized the vesicle fusion site and detected a SNARE conformational change preceding the fusion pore opening in SCORE2 overexpressing SNAP25 KO mouse chromaffin cells. The single fluorescent molecule calibration allowed us to determine the SCORE2 intensity at the releasing site and to further estimate the number of endogenous SNAP25 copies in wild type cells by immunostaining experiment. The determined FRET ratio and apparent FRET efficiency of individual events lead to the estimation of the fraction of SCORE2 molecules exhibiting FRET transition from low FRET state to high FRET state. By extrapolating the results obtained in SCORE2 overexpressing cells, we estimate that in wild type cells ~6-7 SNAP25 molecules undergo conformational change at the fusion site preceding fusion pore opening.

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### P-040. SNARE mediated fusion pore - mechanism and nature

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Fusion of synaptic vesicles to the cell membrane is mediated by SNARE proteins residing on the vesicle and plasma membrane and constitute the minimal fusion machinery. The zippering of the SNARE protein synaptobrevin 2 (syb2) on the vesicle with the t-SNARE proteins, syntaxin-1 (stx-1) and SNAP-25, on the plasma membrane forms a coiled-coil complex. Employing coarse-grained molecular dynamics, simulations of SNARE-mediated fusion of a ~13nm nanodisc with a multicomponent asymmetric planar bilayer is studied. The transmembrane domain (TMD) of syb2 resided in the nanodisc membrane with the t-SNARE complex (stx-1A and SNAP-25) positioned in the planar bilayer by the stx-1TMD and SNAP-25 lipid anchors. Starting with four partially unzipped SNARE-complexes bridging the nanodisc and the planar bilayer, simulations were done to gain an understanding of the fusion mechanism. The simulations revealed that zipping of SNARE complexes pulls the syb2 C terminus deeper into the membrane inducing local curvature at the distal leaflets. The TMD polar residues Y113, S115 and T116 of syb2 and S281, T 282 and G288 of stx-1 form a hydrophilic core between the two distal leaflets facilitating lipid mixing and leading to fusion pore formation. In all of twelve 2.5 us long independent simulations lipid mixing of the distal leaflets with transient fusion pore formation was observed. Four simulations resulted in formation of a stable water-filled pore. The estimated conductances of the simulated fusion pores are in good agreement with experimental values. Two SNARE protein mutations inhibiting fusion experimentally produced only proximal leaflet lipid mixing and no fusion pore formation in the simulations.

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## Poster Presentations

### P-041. FRET-assisted structure prediction of carbohydrate-binding module (CBM56)

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As part of the Critical Assessment of Structure Prediction (CASP13) experiment, the structure of the CASP13 target T0964 (CBM56), the carbohydrate-binding module of a b-1,3-glucanase, should be predicted. The structure information is obtained by measuring distances between different FRET pairs using multiparameter fluorescence spectroscopy, which complement the computational structure prediction based on the amino acid sequence. The experiment includes FRET pair prediction using NMSim coarse-grained simulations to obtain the most meaningful dye positions, the method of gene expression and protein purification to produce the target protein, the performance of the FRET measurements and the analysis of the data for highly accurate structure modeling.

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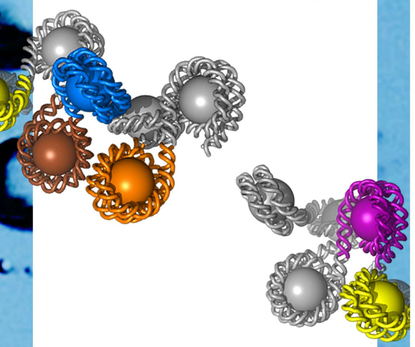



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### P-042. Accurate determination of the RNA three-way junctions via single-molecule high-precision FRET measurements

Olga Doroshenko[1], Hayk Vardanyan[1], Sascha Fröbel[1], Stanislav Kalinin[1], Simon Sindbert[1], Oleg Opanasyuk[1], Christian Hanke[1], Sabine Müller[2], Holger Gohlke[3], Claus A. M. Seidel[1]

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Non-protein coding RNAs are involved in many cellular processes. They commonly exhibit junction motifs and bulges as main architectural building blocks of RNA tertiary contacts which determine molecule's conformations. However, the knowledge about equilibrium structures, conformational space and tertiary conformational changes of large RNAs, being not restrained by external or tertiary interactions, is rather limited.

Förster-Resonance-Energy-Transfer (FRET) restrained high-precision structural modeling is a powerful tool for analyzing the biomolecular structure. We apply multi-parameter fluorescence detection (MFD) of single molecules and ensemble Time-Correlated Single Photon Counting measurements (eTCSPC) to perform FRET study on RNA three- and four-way-junctions (4WJs and 3WJs).

We have generated a database of different RNA 3WJs with different bulges and sequences to study the influence of these factors on the junction conformations for RNA 3WJs. Overall 283 FRET pairs were measured with single-molecule MFD and analyzed with the analysis toolkit [1] that includes probability distribution analysis (PDA) for FRET distance determination and FRET position and screening (FPS) toolkit for structural model generation.

In order to study the influence of the junction on the RNA structures we studied the functional junction part with prolonged helices. Bulge and sequence variations were considered as dominant factors influencing junction conformations for RNA 3WJ. Six different RNA 3WJ with different sequences were studied, two of which have two and five unpaired nucleotides in the junction region.

Furthermore we report that bulges in the junction region determine orientation and rotation of helices, inducing coaxial stacking. Our results show that small changes in the sequence make changes in RNA 3WJ tertiary structures which are expected to have significant impact on the functionality.

[1] Kalinin, S. et al, Nature Methods, 9, 1218–1225 (2012)

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### P-043. β-Wrapin AS69 achieves substoichiometric inhibition of α-synuclein amyloid formation by interference with nucleation processes

Laetitia Heid[1], Emil D. Agerschou[1], Patrick Flagmeier[2], Celine Galvagnion[1,2,3], Christopher M. Dobson[2], Dieter Willbold[1,4], Alexander K. Buell[1,2], Wolfgang Hoyer[1,4]

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β-Wrapins are engineered β-hairpin-binding proteins. β-Wrapin AS69 stabilizes a β-hairpin of α-synuclein comprising amino acid residues 37-54. Our experimental strategy allowed to probe the influence of AS69 on the individual steps of α-synuclein amyloid fibril formation. β-Wrapin AS69 inhibits amyloid formation of α-synuclein at low, substoichiometric concentrations. We find that both growth and proliferation of α-synuclein fibrils are inhibited by AS69. The highly substoichiometric efficiency, which suggests an efficient interference with nucleation processes, is remarkable for a monomer-sequestering agent. To test if the substoichiometric inhibition is solely a consequence of sequestration of α-synuclein monomers by AS69, or if the formed AS69:α-synuclein complex is an active inhibitory species, we created a fusion construct in which AS69 is directly linked to α-synuclein, i.e., a β-wrapin molecule that is already occupied by a α-synuclein monomer. Our results show that the fusion construct, while having little effect on fibril elongation, is able to inhibit aggregation of α-synuclein at substoichiometric concentrations in a secondary nucleation assay. In conclusion, β-wrapin AS69 interferes with crucial molecular processes on the pathway from soluble to fibrillar α-synuclein fibrils. It blocks fibril elongation by sequestering monomeric α-synuclein, and – in the α-synuclein-bound state - achieves inhibition of secondary nucleation.

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### P-044. Using a combination of different labeling techniques to investigate the ras dimer interface via electron spin resonance spectroscopy and FRET-experiments

Christian Teuber[1], Johann Klare[2], Klaus Gerwert[1], Carsten Kötting[1]

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The small GTPase Ras is a crucial intracellular switch regulating cell proliferation and cell growth. Its malfunction is a major factor for cancer development. In 2012 we demonstrated that N-Ras forms dimers at POPC membranes and suggested a potential interface. Hancock and others demonstrated that Ras proteins form clusters at the membrane in order to transmit a signal downstream towards the nucleus. Thus it could be suggested that the Ras dimerization drives the cluster accumulation. Here we want to elucidate the Ras dimer interface at a membrane with EPR- and ATR-FTIR-FRET experiments. For this purpose it is necessary to modify the protein with a c-terminal lipid anchor and with spin-labels or fluorophores at three different sites of interest to obtain distance information for triangulation.

Prior, Ian A., and John F. Hancock. “Compartmentalization of Ras proteins.” Journal of cell science 114.9 (2001): 1603-1608
Güldenhaupt, Jörn, et al. “N-Ras forms dimers at POPC membranes.” Biophysical journal 103.7 (2012): 1585-1593

The first modification was achieved using a maleimido functionalized synthetic lipid anchor reacting with the cysteine 181 of N-Ras. This modification was achieved with nearly 100 % efficiency. The EPR spin-label maleimido-proxyl was attached with maleimide chemistry at C118. Additionally we were able to introduce alkyne groups for click-chemistry into N-Ras using incorporation of the unnatural amino acids ScO and PrK by expansion of the genetic code in combination with a specific aminoacyl-tRNA-Synthetase/tRNA pair. The spin-label azido-proxyl and the FRET-dye Atto532 azide were attached with strain promoted alkyne-azide cycloaddition (SPAAC) at specific positions in N-Ras. The first EPR measurement indicates the suggested interface. Further experiments will be done with copper catalyzed azide-alkyne cycloaddition (CuAAC) in order to achieve a good signal-to-noise ratio in DEER-distance measurements by increasing the label efficiency.

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## Poster Presentations

### P-045. Multiple co-existing structures of an RNA four-way junction resolved by FRET, SAXS, and integrative modeling

Christian A. Hanke[1], Hayk Vardanyan[1], Simon Sindbert[1], Stanislav Kalinin[1], Mykola Dimura[1], Tomasz Soltysinski[2], Grzegorz Lach[2], Danilo Springstubbe[3], Bettina Apel[3], Edward Snell[4,5], Thomas D. Grant[5], Jan Lipfert[6], Sabine Müller[3], Janusz M. Bujnicki[2,7], Holger Gohlke[1], Claus A.M. Seidel[1]

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[6]LMU, München, Germany
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Helical junctions are fundamental structural building blocks of functional RNAs and a major determinant of their architecture. However, RNA folding is often influenced by tertiary interactions with internal (e.g., loops, flexible ends) or external partners (e.g., proteins). The determination of the structure of free helical junctions, in solution and unrestricted by other elements, could yield insights into intrinsic structural features of RNA junctions.
We combined a large number of high-precision FRET measurements with FRET-restrained structural modeling, molecular simulations and SAXS measurements in a hybrid approach to obtain structural models of an RNA four-way junction derived from the hairpin ribozyme. Our integrated modeling approach allowed us to determine three co-existing conformers of the RNA four-way junction molecule with a precision between 1.3 and 3.0 Å. In order to unambiguously identify the minor conformers, we employed their unique $Mg^{2+}$ affinity. This novel identification procedure could also be employed for other molecules than $Mg^{2+}$ ions. We demonstrated that the presented FRET-based hybrid approach is applicable to obtain structural models of large dynamic biomolecules, which proved difficult to study using other techniques.

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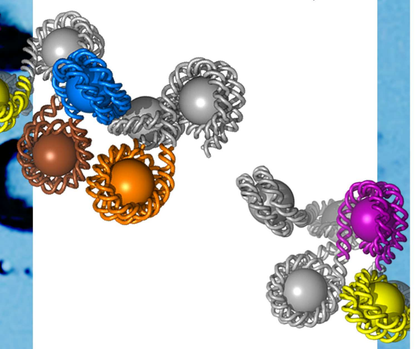



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### P-046. Conformational study of NpSRII/NpHtrII in different lipid nanoparticles using DEER and Rotamer Analysis

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NpSRII/NpHtrII is a transmembrane signaling complex from Natronomonas pharaonis archaea and plays a key role in its negative phototaxis. Structural changes of NpSRII[1] are induced by photon absorption and movements are further conducted to the transducer NpHtrII. The transducer in turn regulates the phosphorylation level of a bound histidine kinase in the cytoplasm that modulates the rotation of the flagellum. Structural information in terms of X-ray crystallographic data for the transmembrane part of this protein complex show two variants: V- and U-shapes[2-3], in addition a model for the complex with an extended transducer is available[4].

This work aims to study the conformation of the NpSRII/NpHtrII complex in different cell membrane mimicking environments. Interspin distance distributions were determined by pulse EPR experiments (DEER) and compared with in silico spin-label rotamer analyses obtaining distance distributions between two spin labels in the cytosolic side of the protein complex. This analysis was performed using MMM[5] and MtsslWizard[6] software. The results show some differences in the conformations of the protein complex in SMALPs and nanodiscs[7], which are discussed in relation to the different crystal structures using the rotamer simulations.

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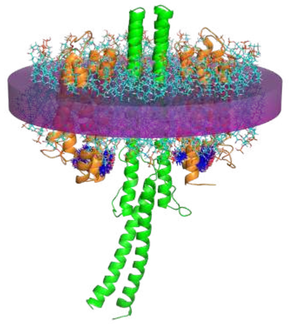

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### P-047. Integrative single-molecule FRET analysis of multistate conformational dynamics

Oleg Opanasyuk[1], Hugo Sanabria[2], Thomas-Otavio Peulen[1], Suren Felekyan[1], Stanislav Kalinin[1], Claus Seidel[1]
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Single-molecule Förster Resonance Energy Transfer (smFRET) experiments in Multiparameter Fluorescence Detection (MFD) mode contain a wealth of information regarding the temporal evolution of labeled biomolecules, proteins or nucleic acids a like. Capturing exchange rate constants among the polymorphic configurations of biomolecules is a major challenge in smFRET experiments. We present a set of parametric relationships between FRET indicators, called FRET lines. FRET lines serve as gridlines to understand and interpret biomolecular dynamics in two-dimensional MFD histograms and help us decouple the motions of the fluorescence labels from their hosts. To provide a holistic, self-consistent analysis of smFRET experiments across various data representations, we introduce an integrative single-molecule analysis of kinetics that jointly satisfies FRET lines, Time Correlated Single Photon Counting (TCSPC), Fluorescence Correlation spectroscopy (FCS) and Photon Distribution Analysis (PDA). At first 2-dimensional burst frequency histograms for the two FRET indicators, fluorescence weighted average donor fluorescence lifetime and intensity-based FRET efficiency, are computed for each experiment. A visual (qualitative) analysis includes the following steps: 1) Determine the number of FRET species and classify the populations as static or dynamic by computing model specific FRET-lines; 2) Identify the FRET species mixed in dynamic populations by sub-ensemble analysis (TCSPC, FIDA); 3) Identify the connectivity of FRET species by computing dynamic FRET-lines; and 4) Estimate the exchange rate constants from the peak shapes and by color- and species-fluorescence correlation analysis. This information can be applied as hypotheses in the subsequent quantitative analysis. Individually, the presented methodologies provide ambiguous results for complex kinetic networks; however, together, reaction rate constants and the connectivity in multi-state dynamics systems can be resolved.

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### P-048. Disulfide bond formation between two amyloidogenic regions of islet-amyloid polypeptide inhibits amyloid fibril formation

Tatsiana Kupreichyk[1,2], Wolfgang Hoyer[1,2], Lothar Gremer[1,2], Dieter Willbold[1,2]

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Abnormal protein folding or aggregation of normally soluble proteins into amyloid fibrils has become a subject of fundamental relevance, as it arises in many widely occurring age related diseases, ranging from Parkinson"s and Alzheimer"s diseases to spongiform encephalopathy and Type 2 Diabetes. Better understanding of mechanisms of misfolding and aberrant protein aggregation is needed to prevent or inhibit development of the disease.

Islet amyloid polypeptide (IAPP, also called Amylin) aggregates into toxic oligomers, amyloid fibrils and later into deposits in islets of Langerhans in pancreas, and these aggregates are believed to cause β-cell dysfunction in Type 2 Diabetes. IAPP contains 37 amino acid residues and has a random coil conformation in its native state. However, it tends to build amyloid aggregates due to the presence of amyloidogenic regions, which are prone to adopt β-sheet structure. Consequently, the transition from an intrinsically disordered monomeric state into β-sheet-rich fibrils may happen and lead to pathology.

Here we introduce IAPP-CC, which is IAPP with two substituted amino acids: Ala13 and Ala25 are replaced by two cysteines. This replacement is meant to lead to formation of an additional disulfide bridge and to stabilize IAPP against amyloid fibril formation. Recorded circular dichroism (CD) and nuclear magnetic resonance (NMR) spectra have shown that this double-cysteine mutant of IAPP has the desired structure: the newly-introduced cysteines form a disulfide bridge between each other and apparently do not affect the natural one between Cys2 and Cys7. Aggregation kinetics followed by Thioflavin T (ThT) fluorescence have shown that IAPP-CC is more stable, as it exhibits a longer lag phase and slower aggregation rate. The presence of IAPP-CC has a well-defined inhibitory effect on wildtype IAPP aggregation. The combination of kinetic data with morphological study of aggregates reveals that IAPP-CC effectively hinders fibril elongation.

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## Poster Presentations

### P-049. Accuracy in FRET measurements concerning technical and methodical aspects

Julian Folz[1], Thomas Peulen[1], Oleg Opanasyuk[1]
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Fluorescence spectroscopy and imaging are important biophysical techniques to study dynamics and function of biomolecules in vitro and in live cells. The use of more than one fluorophore per molecule opens additional opportunities arising from photon densities, coincidences and dipolar coupling by Förster Resonance Energy Transfer (FRET) to study the stoichiometry and structure of biomolecular systems. Before performing FRET measurements one needs to consider which data should be acquired and what information is contained in the data with what accuracy. We compared data of fluorescence lifetime and fluorescence intensity experiments for the sake of FRET based-structural integrative modelling though dye models. Different models of the fluorescent dyes used to model observables of FRET experiments will be introduced and discussed concerning their accuracy. Exploiting the solvatochromism of fluorescent dyes in time-resolved fluorescence experiments, we probe experimentally fully solvated dyes and dyes bound to protein surfaces. Such data will be presented for a network of labeling sites of a large GTPase and may be utilized for refined coarse-grained dye models for high-speed integrative structural modelling at high accuracy.

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### P-050. Mobility-based quantification of virus-lipid interactions

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Multivalent interactions, i.e., multiple, non-covalent ligand-receptor bonds acting in parallel, are typical for a multitude of biological processes. They are observed, for example, in the attachment of viruses to the membrane of their host cells, a dynamic process that eventually leads to internalization and infection. Rational design of strategies to prevent infection requires quantification of the underlying multivalent interaction (e.g., in absence and presence of antivirals). This is still an unresolved problem as the individual ligand-receptor association is typically weak and accordingly each bond is maintained only transiently, causing temporal fluctuations of the valency (i.e., of the number of engaged bonds). Quantification of multivalent interactions therefore requires methods offering single particle (e.g., virus or pathogen) resolution, since only in this way heterogeneities and dynamics, which are typically hidden in conventional ensemble-averaging approaches, can be scrutinized. Motivated by our previous work on bilayer-tethered liposomes, we estimate here the valency of single influenza A/X31 viruses (interacting with the ganglioside GD1a) by measuring virus mobility using total internal reflection fluorescence microscopy (TIRF) and single particle tracking (SPT), allowing the complex off-rate distribution to be deconvoluted from valency effects. Application of this approach to quantify changes of multivalent virus-lipid interactions caused by addition of virus inhibitors will be discussed.

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### P-051. Anisotropic metal growth on phospholipid nanodiscs via lipid bilayer expansion

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

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## Poster Presentations

### P-052. Microviscosity of bacterial biofilm matrix characterized by fluorescence correlation spectroscopy and single particle tracking

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Bacterial biofilms are surface-adherent communities of bacteria surrounded by an extracellular polymeric substance (EPS) consisting of secreted polysaccharides and other biomolecules. In healthcare settings, bacterial biofilms represent a severe threat causing chronic infections and contamination of medical devices. To remove biofilms, multiple strategies have been developed, e.g. treatment with antibiotics or bacteriophages, which are advantageous to specifically target bacterial species. However, it remains unclear to which extent the EPS matrix imposes a physical barrier for the transport of bacteriophages to the targeted bacteria. To address this question, we have reconstituted the EPS matrix of the bacterium Pantoea stewartii, responsible for a severe disease of corn plants, and investigated the diffusion properties of differently sized fluorescent particles, using fluorescence correlation spectroscopy and single particle tracking. This approach allows the study of the EPS matrix spatial organization under various physico-chemical conditions (pH, salt content, ion concentration). We show that small probes diffuse unrestrained in the EPS with diffusion coefficients similar to those measured in water. In contrast, larger probes are drastically slowed down, showing anomalous subdiffusion. The degree of confinement increases with the EPS concentration. At physiological concentrations, beads of the size of bacteriophages are up to 50-fold slowed down compared to the dynamics observed in aqueous solution. To overcome the physical barrier imposed by the EPS, bacteriophages are equipped with EPS degrading enzymes. By treating the EPS with purified bacteriophage enzymes, we show that strongly confined diffusion turns to free diffusion within few minutes. Thus, our approach allows the investigation of dynamic changes in bacterial biofilm matrix microviscosity and shows that the EPS imposes a strong, probe-size dependent diffusion barrier under physiological conditions.

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### P-053. Conformational changes of channelrhodopsin-2 investigated by time-resolved EPR spectroscopy

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Channelrhodopsin-2 is a cation-selective light-gated channel[1,2], which is observed as a dimer. Its manifold usage has established channelrhodopsin-2 as the most prominent optogenetic tool[3]. 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[4].

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[4,5,6]. Mutants, which were available for distance measurements, were used here for time-resolved EPR spectroscopy to provide information about the relation between transitions of the photocycle, the helix movements, and channel formation.

The light-minus-dark difference continuous wave EPR spectrum with spin labels on helix B and helix F and the time constant for the light induced conformational change could be resolved. Furthermore, the origin of the difference spectra could be explained, utilizing EPR spectra simulations, by a transient increase of the mobility of the spin label. These results relate the observed light induced transient movement of helix B, its coupling to channel opening and closing[3], to transitions between photocycle intermediates.

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### P-054. Influence of aggregation partners on the secondary structure of peptides

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A variety of biological examples exist in which peptide-metal interactions play a decisive role (e.g. ion channels, active centres of proteins). The influence of metal cations like copper, zinc iron, calcium or aluminium was also discussed for the aggregation of pathogenic peptides in neurodegenerative diseases like Alzheimer"s, Parkinson"s or Creutzfeldt-Jakob disease. Thus the study of the impact of metal cations on amino acids and small peptides is a first logic step within a bottom-up approach. In this context we started investigations on the attachment of singly (and partly triply) charged aluminium cations to the protected amino acids Phe, Tyr, Trp, Val or Ala. In a spectroscopic bottom-up approach, analysing intrinsic structural properties and aggregation behaviour, IR(MPD) as well as IR+UV photodissociation spectroscopy in a molecular beam was applied. By comparison with DFT calculations structural assignments were performed.

Another environmental factor that influences the backbone structure of a peptide is the attachment of a second peptide, which (as mentioned above) can also be of interest with regard to neurodegenerative diseases. Based on our former studies on different peptide homo dimers we now analysed a hetero dimer consisting of the tripeptide model Ac-Val-Tyr(Me)-NHMe and the tripeptide Ac-Ala-Ala-Ala-OMe by combined IR/UV molecular beam spectroscopy. For comparison the single components were also structurally investigated with the Ala containing tripeptide being analysed by temperature dependent gas phase FT-IR spectroscopy (due to the missing UV chromophore for combined IR/UV spectroscopy). It could be demonstrated that the Ac-$(Ala)_3$-OMe structurally rearranges when aggregated to the Ac-Val-Tyr(Me)-NHMe peptide so that finally the hetero dimer forms an antiparallel β-sheet arrangement.

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### P-055. Microfluidic diffusional sizing enables characterisation of Protein-Protein interactions under native conditions

Maren Butz[1], Maya Wright[1,2], Jonathan Faherty[1], Tom Scheidt[2], Jackie Carozza[2], Paolo Arosio[2,3], Emma Yates[2], Yuewen Zhang[2], Alex Buell[2], Thomas Müller[1,2], Sean Devenish[1], Chris Thorne[1], Tuomas Knowles[1,2]

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The characterization of biomolecular interactions is a field with applications in health research and diagnostics, yet few current methods are applicable to studies taking place in physiologically-relevant conditions or where sample volumes are limited. Here we discuss a powerful approach for probing the sizes of proteins and their complexes in solution - microfluidic diffusional sizing (MDS). Importantly, with MDS small volumes of protein are studied with high sensitivity in their native state without the use of a matrix or surface.

The system is maintained at steady state laminar flow, which enables the diffusion of protein species as they pass through a microfluidic channel to be measured, and consequently their hydrodynamic radius (Rh) to be determined.

Preliminary experiments show that this approach has potential applications ranging from basic biophysical analyses to in-solution characterization of protein-protein interactions (PPIs) and the formation of protein aggregates. We are developing therefore a range of products that will make MDS available to any lab wishing to use it.

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## Poster Presentations

### P-056. The Deutsche Forschungsgemeinschaft at a glance-research funding opportunities

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The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) is the largest independent research funding organisation in Germany. It serves all branches of science and the humanities by funding research projects at universities and other research institutions.

The DFG promotes excellence by selecting the best research projects on a competitive basis and facilitating national and international collaboration among researchers. Its mandate also includes encouraging the advancement and training of early career researchers, promoting gender equality in the German scientific and academic communities, providing scientific policy advice, and fostering relations between the research community and society and the private sector.

In this contribution, a selection of DFG funding programmes will be presented at a glance, putting a special emphasis on funding opportunities for early career scientists. During the poster session, there will be room to answer your questions and to discuss some “dos and don”ts” when preparing a funding proposal with a member of the DFG head office.

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### P-057. High precision FRET studies reveal reversible transitions in nucleosomes on the microsecond to minute time scale

Ralf Kühnemuth[2], Alexander Gansen[1], Suren Felekyan[2], Kathrin Lehmann[1], Katalin Tóth[1], Claus A.M. Seidel[2], Jörg Langowski[1]

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Nucleosomes play a dual role in compacting the genome and regulating the access to DNA. To unravel the underlying mechanism, we studied fluorescently labeled mononucleosomes by multiparameter FRET measurements and characterized their structural and dynamic heterogeneity upon NaCl-induced destabilization. Species-selective fluorescence lifetime analysis and dynamic photon distribution analysis revealed intermediates during nucleosome opening and led to a coherent structural and kinetic model. In dynamic octasomes and hexasomes the interface between the H2A-H2B dimers and the (H3-H4)2 tetramer opens asymmetrically by an angle of ≈20° on a 50 and 15 µs time scale, respectively. This is followed by a slower stepwise release of the dimers coupled with DNA unwrapping. A mutation (H2A-R81A) at the interface between H2A and H3 facilitates initial opening, confirming the central role of the dimer:tetramer interface for nucleosome stability. Partially opened states such as those described here might serve as convenient nucleation sites for DNA-recognizing proteins.

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### P-058. Thermodynamic analysis of the hydration layers of bio-active molecules and proteins

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Hydration has a major impact on structure, stability and function of proteins. Protein stability and defined structural integrity is fundamentally based on essentially weak interactions between peptide surfaces competing against interactions with the solvent and co-solutes. Enthalpy/entropy compensation is the key for this process, which is still not understood in quantitative terms. A large variety of physical and molecular parameters contribute to the two scale pans (pro or contra folding) like polarity, viscosity, H-bonding propensity, up to molecular crowding and confinement.

In the center of interest of this study is the change in the Gibbs free energy of the available hydration water which differs from the bulk water, in particular in dependence of co-solute´s type and concentration. The latter defines the number of solvation layers formed.

For determination of the change in energies of solvation of lysozyme and various co-solutes like NaCl, betaine and urea, isothermal titration calorimetry was applied to define the change in enthalpy and microscale thermophoresis for the change in entropy of hydration [1-2].

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### P-059. Time-resolved IR spectroscopic studies of Channelrhodopsin-1 and cysteine variants

Raiza Maia[1], Maria Walter[1], Ramona Schlesinger[1], Joachim Heberle[1]

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Channelrhodopsins (ChRs) belong to a branch of the microbial rhodopsin family, which can be found in green algae acting as phototaxis receptors. ChRs are still the only light-gated ion channels found in Nature. This functionality founded the basis of optogenetics, While Channelrhodopsin-2 from Chlamydomonas reinhardtii (CrChR2) has been widely studied and mostly used optogenetic ChR, little is known about the structural dynamics of Channelrhodopsin-1 from Chlamydomonas augustae (CaChR1). Therefore, this work presents investigations on wild-type CaChR1 and its mutants. It covers the photocycle kinetics with focus on the cysteine S-H stretching region that has been analyzed by time-resolved IR difference spectroscopy employing novel quantum cascade lasers.

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### P-060. Kinetic and thermodynamic aspects of fibrils elongation

Nicola Vettore[1], Lena Mangels[1], Alberto Coden[1], Alexander K. Buell[1]
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Amyloid fibrils are non-covalent protein polymers with extraordinary thermodynamic stability. While the high thermodynamic stability of this polymeric state of proteins is widely known and well-established, not many efforts have been directed towards a quantitative understanding of the physical origin of this stability.

An experimental strategy that can be applied to quantify the stability of folded proteins as well as protein assemblies is equilibrium denaturation, which involves the use of chemical denaturants such as Urea and GdnCl to modify the difference in free energy between the states. Applying this method to the characterisation of amyloid stability is possible if the amyloid fibrils in equilibrium with soluble protein behave as a two state system, with no (equilibrium) intermediates between monomeric and amyloid states.

Here I present a detailed thermodynamic and kinetic study of an amyloid fibril-forming protein, the PI3K-SH3 domain. The initial aim is to characterize the system under a range of solution conditions and temperatures within which we can establish the two-state approximation to be valid, followed by a more detailed analysis of the elongation reaction. The experimental data I will present are mostly based on quartz crystal microbalance (QCM) and intrinsic fluorescence measurements, which are used to study the kinetic and the equilibrium behavior of the fibrils, respectively.

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### P-061. Impact of co-solutes on the interaction of the intrinsically disordered transcription factor c-Myb and its interaction partner CBP

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Intrinsically disordered proteins (IDPs) are characterized by a partial or global lack of secondary structure elements under physiological conditions, which allows them to bind to their interaction partner with low affinity and high specificity. A common feature among IDPs is a folding upon binding process, during which the IDP samples through its funneled energy landscape in search for favorable intra- and/or intermolecular interactions, which are stabilizing the final complex. This structural plasticity enables IDPs to play key roles in cellular signaling and regulatory pathways. However, thermodynamics, kinetics and binding mechanisms of IDPs are poorly understood.

Goal of this work is to study the impact of solvent and co-solutes on the interaction of the intrinsically disordered transcription factor c-Myb and its interaction partner CREB-binding protein (CBP). We use different techniques, including temperature jump relaxation and microscale thermophoresis to study both kinetics and thermodynamics of the interaction. By applying a variety of co-solutes, such as osmolytes, electrolytes, artificial polymer crowders as well as protein crowders like BSA and Lysozyme we are able to quantify the effect of each co-solute on the interaction of c-Myb and CBP. Further work will include a mutagenic study, revealing the impact of the solvent on near-residue level.

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### P-062. The impact of macromolecular crowding on translational mobility and conformational properties of proteins

Alyazan Albarghash[1], Niklas Junker[1], Henning Höfig[1], Daryan Kempe[1], Julia Walter[1], Julia Otten[2], Martina Pohl[2], Simone Wiegand[2], Jörg Fitter[1,2]

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Studies on various protein characteristics are typically performed in-vitro under conditions that deviate significantly from the natural surrounding conditions within the densely packed cytoplasm of the living cell. Therefore, the results of in-vitro experiments may represent the special properties of the protein under the explicitly given in-vitro conditions and not by necessity those properties naturally exhibited in the living cell. Despite advancements in live-cell fluorescence microscopy and spectroscopy, the techniques of labeling biomolecules inside the cell remain limited to a few biomolecules species. A way to approach this problem is by mimicking the crowded cellular cytoplasm with artificially crowded microenvironments. The effect of macromolecular crowding on the translational mobility of the proteins is studied with Fluorescence Correlation Spectroscopy (FCS). Further, crowding has a pronounced effect on the conformational properties of intrinsically disordered proteins and multimeric protein complexes. These effects are studied by conducting Förster Resonance Energy Transfer (FRET) on FRET-based multimeric biosensors. Experiments on differently shaped and sized proteins in diversely crowding conditions are conducted. FCS measurements show a reduction of the translational mobility of different proteins and a systematic deviation from Brownian diffusion upon the introduction of crowding agents to the solution. Further, FRET measurements reveal a systematic increase in the compactness of FRET-based metabolite biosensors and crowding biosensors with the crowder concentration in the surrounding microenvironment.

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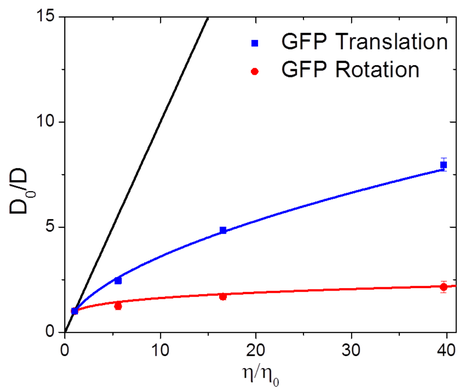


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### P-063. An improved two-color coincidence detection for quantifying the interlinkage of macromolecular components at single molecule level

Henning Höfig[1,2], Cristina Remes[3], Noémie Kempf[4], Alexandros Katranidis[2], Daryan Kempe[5], Jörg Fitter[1,2]

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Intermolecular binding between different biomolecules and the state of association in macromolecular complexes is a crucial issue in many biological processes. Direct quantification of how many diffusing molecules in a solution are really bound to each other would be a valuable tool to characterize the functional performance of involved interaction partners.

Two-color coincidence detection (TCCD) is based on the attachment of fluorophores of different colors to each of the individual binding partners. TCCD requires a dual-color detection of single molecules. A coincidence analysis of both fluorescence signals reveals whether the interaction partners are bound (i.e. they diffuse together) or not. However, conventional confocal TCCD suffers from an underestimation of coincidence.

Here, we introduce an improved TCCD analysis that makes use of an additional acceptance criterion for the individual fluorescence signals. First, we demonstrate the capabilities of our method using different DNA-based reference samples. Subsequently, we quantify the extent of chromophore maturation of fluorescent proteins in genetically encoded FRET-based biosensors. In a second application we unravel a basic mechanism in bacterial protein synthesis initiation. According to text book knowledge, protein synthesis initiation requires dissociated 30S and 50S ribosomal subunits before translation and chain elongation of a new protein can start. By employing our TCCD method we verifiy that proteins synthesis is also initiated directly by 70S complexes (i.e. 30S and 50S subunits remain associated). We found that 38% of translation is initiated directly by 70S complexes.

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## Poster Presentations

### P-064. Interaction network of apoptotic Bcl-2 proteins addressed by EPR and ODNP techniques

Svetlana Kucher[1], Mohammad Hadi Timachi[1], Tufa Enver Assafa[1], Markus Teucher[1], Sukhendu Nandi[1], Laura Galazzo[1], Stephanie Bleicken[1], Enrica Bordignon[1]

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Site-directed spin labeling combined with EPR spectroscopy is a powerful biophysical tool to monitor conformational changes and interactions of biomolecules. Here we show how to combine continuous wave EPR and pulsed dipolar spectroscopy (DEER) with a newly developed tool, namely Overhauser Dynamic Nuclear Polarization (ODNP) to obtain site-specific information about dynamics, interspin distances and water accessibility.

ODNP can be performed on minimal sample volume (few microliters) at physiological temperature, and allows a direct measurement of water accessibility of a spin-labeled side chain with no need of freezing the sample, or adding paramagnetic quenchers.

We will present the ODNP setup in our lab and first results on the interactions between proteins of the Bcl-2 family during apoptosis initiation and inhibition. In particular, we will study the inhibition process of the pro-survival Bcl-XL protein and the regulation of inhibition and activation of the pro-apoptotic Bax into mitochondrial outer membrane (MOM) by BH3-only proteins like BIM peptides and c-Bid. Moreover, orthogonal labeling strategies will be presented, which allow to address specifically different proteins in the same sample, in terms of dynamics, distances and also water accessibility.

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## Poster Presentations

### P-065. Picosecond pulses with wavelength freedom from UV to nIR

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In recent years, time resolved techniques such as Time Correlated Single Photon Counting, Fluorescence Lifetime Imaging Microscopy or Diffuse Optical Tomography have shown a huge potential to study biophysical samples in a minimal invasive fashion while obtaining high contrast. These techniques rely on short laser pulses, typically < 100 ps. However, conventional pulsed Lasers are limited in output power and to certain wavelengths due to the available gain mediums. This limitation can be circumvented by fiber laser technology and the nonlinear process of supercontinuum generation in highly nonlinear photonic crystal fibers. Here we show how state of the art fiber lasers can deliver any wavelength from 270 - 2400 nm at fairly high output powers and short picosecond pulse durations.


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### P-066. hGBP 1 – polymerization and membrane binding is more than just a farnesyl switch

Linda Sistemich[1], Christian Herrmann[1]

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Human guanylate-binding proteins (hGBPs) belong to the dynamin superfamily of large GTPases and they are upregulated in cells as an immune response against microbes and viruses. Three of the seven isoforms contain a C-terminal CaaX motif which can be isoprenylated. In the cell, the isoprenyl anchor allows the hGBPs to attach to organelle and plasma membranes. In vitro it was shown that the nucleotide dependent binding to artificial membranes is competed with a GTP dependent polymerization leading to tube-like structures. It is mandatory to expose the farnesyl tail (farnesyl switch) for both actions. To gain a better understanding of the cellular functions of hGBP 1, we tried to unscramble the interaction mechanism of farnesylated hGBP 1 (hGBP 1fn). Therefore, fluorescence microscopy and spectroscopy, an absorption-based turbidity assay and hydrolysis assays were performed. We showed that polymerization as well as membrane binding can be altered by introducing different point mutations to hGBP 1. These point mutations lead to changes in the crucial properties that are needed for the function of the farnesylated protein as GTP hydrolysis, dimer formation or intramolecular rearrangements. With these findings, we gain a more detailed insight into the nucleotide dependent interactions between the hGBPs while polymerization and membrane binding.

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### P-067. Human guanylate binding protein 1 retains its activity while interacting with nonstructural protein 1 from influenza A virus

Ping Zhang[1], Christian Herrmann[1]

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During influenza A virus (IAV) infection, upregulated expression of human guanylate binding protein 1 (hGBP1, localized in the cytoplasm) inhibits the viral replication in a dose-dependent manner. Cellular studies observed direct interaction of hGBP1 with the viral nonstructural protein 1 (NS1). Upon upregulation of hGBP1, NS1 is translocated from the nucleus to the cytoplasm. In vivo studies claim that NS1 eliminates enzymatic activity of hGBP1 and the expression of NS1 in healthy human cells decreases the cells resistance against IAV infection. Together, it was considered that NS1 antagonizes the antiviral activity of hGBP1. [1]

In this work, we characterized the interaction between hGBP1 and NS1 in vitro, quantifying the equilibrium dissociation constant ($K_d$) in micro molar range. We examined the interaction with different domains of hGBP1 and discovered that hGBP1 might expose more than one binding site for NS1 due to its large conformational change during GTP/GDP hydrolysis. Further, we found that the interaction with NS1 does not antagonize the GTPase activity of hGBP1. Properties of hGBP1 such as nucleotide binding, dimerization and hydrolysis, were not altered by the interaction. In summary, we confirmed that hGBP1 directly binds to NS1 but we observed no impact of NS1 interaction on the properties of hGBP1.

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### P-068. Single-molecule FRET experiments for investigation of PARP-1 binding mechanism to DNA single-strand breaks

Anna Sefer[1], Eleni Kallis[1], Tobias Eilert[1], Mara Guariento[1], Nadine Jakobi[1], David Neuhaus[2], Sebastian Eustermann[3], Jens Michaelis[1]

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Single molecule Förster Resonance Energy Transfer (smFRET) experiments for investigation of PARP-1 binding to DNA single-strand breaks were performed on a confocal microscope which combines multiparameter fluorescence detection (MFD) and pulsed interleaved excitation (PIE) techniques. Thus, separation of various subpopulations in heterogeneous samples according to characteristic parameters such as FRET efficiency, lifetime, anisotropy or labeling stoichiometry is possible which makes smFRET experiments on single biomolecular complexes a useful tool for investigation of protein-DNA interactions.

The multidomain enzyme Poly-(ADP-ribose)-polymerase-1 (PARP-1) plays an important role in genome-related processes such as DNA repair and transcription regulation. It is a key DNA damage signaling protein that detects DNA single strand breaks (SSBs). Upon PARP-1 binding to a SSB, the DNA adopts a highly kinked conformation such that the damaged DNA site is exposed to the surroundings. This leads to PARP-1 activation and poly-ADP-ribosylation (PARylation) of various target proteins. PARylation acts as a signal for the DNA repair machinery, which will assemble on the damage site and repair the SSB.

Here we aim to investigate the mechanism of SSB recognition by PARP-1 and especially the associated conformational changes of the DNA on the single-molecule level. To imitate a single strand break, we use a fluorescently labeled DNA ligand with a nick (one phosphate bond missing) in the middle. The fluorescent labels on the DNA are positioned such that different degrees of DNA kinking (induced by PARP-1) lead to different FRET efficiencies depending on different DNA conformations. Thus, we address the mechanistic question, how exactly PARP-1 binds the SSB DNA. Furthermore, we quantify the kinking angles of the DNA for different binding states using a combination of computational approaches and the described smFRET experiments.

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### P-069. Single-molecule FRET Analysis of a Cellulosome Scaffoldin reveals dynamic interactions of type I Cohesin modules

Anders Barth[1], Jelle Hendrix[1,2], Daniel Fried[3,4], Yoav Barak[3,5], Edward Bayer[3], Don C. Lamb[1]

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Efficient degradation of plant cell walls by selected anaerobic bacteria is performed by large extracellular multienzyme complexes termed cellulosomes. The spatial arrangement within the cellulosome is organized by a protein called scaffoldin, which recruits the cellulolytic subunits through interactions between cohesin modules on the scaffoldin and dockerin modules on the enzymes. Although many structural studies of the individual components of cellulosomal scaffoldins have been performed, the role of potential interactions between individual cohesin modules and their flexible linker regions between them are still not entirely understood. We performed single-molecule measurements using Förster resonance energy transfer (FRET) to study the conformational dynamics of a bimodular tandem cohesin segment of the scaffoldin protein CipA of Clostridium thermocellum. Our data reveal the existence of compacted structures in solution that persist on the timescale of milliseconds. This compacted conformation is found to be in dynamic equilibrium with an extended state that shows distance fluctuations on the microsecond timescale. The dynamic interconversion rates are quantified by dynamic photon distribution analysis (PDA) and filtered fluorescence correlation spectroscopy (fFCS). The formation of cohesin-cohesin interactions is also observed in all-atom molecular dynamics simulations of the system. From the simulations, we identify possible inter-cohesin binding modes, none of which show obstruction of the cohesin-dockerin binding interfaces. Our results go beyond the view of scaffoldin as "beads on a string". We propose that both the flexibility and cohesin-cohesin interactions are important factors for the precise spatial arrangement of the enzymatic subunits in the cellulosome that leads to the high catalytic synergy in these assemblies. Hence, the flexibility of the linker region and cohesin-cohesin interactions should be considered when designing cellulosomes for industrial applications.

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### P-070. Azidohomoalanine as site-specific probe for steady state and 2D-IR spectroscopy on an allosteric protein

Katharina B. Eberl[1], Julian Schmidt-Engler[1], Patrick Quoika[1], Christian Bonifer[1], Jan G. Löffler[1], Henrike M. Müller-Werkmeister[1], Martin Essig[1], Jens Bredenbeck[1]

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The non-canonical amino acid Azidohomoalanine (Aha) is an excellent label for IR spectroscopy in proteins that provides local information of a selected site. Its absorption wavenumber and vibrational relaxation dynamics is highly sensitive to the local microenvironment and probes hydrogen-bonding and electrostatic effects. By placing Aha site-specifically in a protein it is possible to monitor even subtle conformational and dynamical changes using steady-state and 2D-IR spectroscopy.

We use Aha to investigate allosteric communication within the 3rd PDZ domain of the synaptic protein PSD-95, which is an extensively studied but controversially discussed model for dynamic allostery without major conformational changes. A network of coupled amino acids, which might be involved in intradomain information transfer and allosteric communication, has been proposed by Lockless and Ranganathan in 1999. Many other theoretical and some experimental studies tried to proof or disproof the existence of a coupled network of amino acids; however, the presence and mechanism of intradomain information transfer still remains unclear. Our approach allows measuring tiny conformational and dynamical changes which have been invisible before. Thus, we are able to detect weak influences of ligand binding, which trigger allosteric effects. We observe absorption band shifts up to 8 cm-1, significant variations in lineshape and changes in vibrational lifetime for different PDZ-Aha mutants. Thereby we are able to get a deeper insight in allosteric communication of the PDZ3 domain.

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## Poster Presentations

### P-071. Diffusion dynamics, activation, and cross-interaction of receptor tyrosine kinases studied by single-particle tracking

Marie-Lena I.E. Harwardt[1], Sebastian Strauss[2,3], Ralf Jungmann[2,3], Marina S. Dietz[1], Mike Heilemann[1]

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Receptor tyrosine kinases (RTKs) contribute to cell proliferation and motility. They react to a variety of ligands, interact with a multitude of cell components and proteins, and initiate complex signaling cascades. Dysregulation causes various diseases including cancer [1]. Successful therapy demands detailed knowledge of receptor activation, cross-reactivity, and molecular interactions. Single-particle tracking provides the required sensitivity to study these transient but crucial cellular mechanisms at the level of single cells and single membrane receptors.

We focused on two RTKs, MET and epidermal growth factor receptor (EGFR), representing prominent members of two different RTK subfamilies that were previously reported to cross-interact, for example in lung cancer cells [2]. We profiled the diffusion behavior of active and resting MET receptors in living cells. Application of a variety of cytotoxins revealed influences of actin, microtubules, and cholesterol on MET diffusion dynamics and endocytosis [3]. We next investigated whether MET and EGFR activation are coupled by stimulating both receptors either with one or a combination of both cognate ligands (EGF, HGF). Analysis of receptor dynamics upon exposure to a ligand yielded information on the extent of cross-activation in different types of cancer cells.

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## Poster Presentations

### P-072. EPR spectroscopy for the analysis of protein-protein interaction in highly concentrated liquid protein solutions

Jacob Blaffert[1], Haleh Hashemi Haeri[1], Michaela Blech[2], Patrick Garidel[2], Dariush Hinderberger[1]

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An increasing number of therapeutic monoclonal antibodies is developed for subcutaneous administration, often requiring protein concentration of more than 100 mg/ml. These high concentration conditions can lead to unwanted solution behaviour, such as phase separation, aggregation and high viscosities, which can impair several steps in the shelf life of the protein. To avoid or suppress this solution behaviour a profound understanding about the mechanistic origins is needed. Protein-protein self-interactions are based on weak, non-covalent forces, such as electrostatic and hydrophobic interactions, and are assumed to play a major role in the formation of concentration-dependent solution behavior. However, common methods for analyzing protein-protein self-interaction, such as light-scattering- and chromatography-based methods, are limited to dilute protein conditions and fail to provide mechanistic insights into the origins of the solution behavior of highly concentrated liquid protein solutions.

In our approach, we explore the utility of electron paramagnetic resonance (EPR) spectroscopy for analyzing protein-protein self-interactions in monoclonal antibody solutions. The effects of antibody concentration as well as formulation conditions (i.e. pH, ionic strength, and temperature) are evaluated by continuous wave EPR and a variety of pulse EPR spectroscopic methods. The study includes experiments with spin-labeled antibodies and the use of different types of soluble spin probes with respect to size, charge and hydrophobicity.

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### P-073. Role of force for the self-assembly of Myosin II minifilaments

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Force generation and self-assembly are two central processes in biological systems that usually are considered in separation. However, the signals that activate non-muscle myosin II simultaneously lead to self-assembly into myosin II minifilaments as well as progression of the motor heads through the crossbridge cycle.

Here we investigate theoretically the possible effects of coupling these two processes. Our assembly model, which builds upon a consensus architecture of the minifilament, predicts a critical aggregation concentration at which the assembly kinetics slows down dramatically.

We validate our model by comparing fluorescence recovery after photobleaching simulated with our model against experimental results.

Our model also predicts that increasing actin filament concentration and force both lead to a decrease in the critical aggregation concentration.

We suggest that due to these effects, myosin II minifilaments in the cell might be in a supercritical state that can react faster to changing conditions than in solution.

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### P-074. Electron cryo-microscopy structure of the canonical TRPC4 ion channel reveals the binding site for its regulatory proteins

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Canonical transient receptor channels (TRPC) are non-selective cation channels. They are involved in receptor-operated Ca2+ signaling and have been proposed to act as store-operated channels (SOC). Their malfunction is related to cardiomyopathies and their modulation by small molecules has been shown to be effective against renal cancer cells. The molecular mechanism underlying the complex activation and regulation is poorly understood. Here, we report the electron cryo-microscopy structure of zebrafish TRPC4 in its unliganded (apo), closed state at an overall resolution of 3.6 Å. The structure reveals the molecular architecture of the cation conducting pore, including the selectivity filter and lower gate. The cytoplasmic domain contains two key hubs that have been shown to interact with modulating proteins. Structural comparisons with other TRP channels give novel insights into the general architecture and domain organization of this superfamily of channels and help to understand their function and pharmacology.

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### P-075. Biomolecular dynamics studied by time-resolved IR-spectroscopy and laser-excited perturbation techniques

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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 whereas perturbation techniques are better suited. Pulsed laser-excitation can be used to generate fast jumps in temperature or in pH and to analyze non-equilibrium relaxation dynamics of proteins. We built-up a spectrometer with tunable quantum cascade lasers (QCL) for mid-IR detection combined with a laser-excited T-jump or pH-jump for the fast (nanosecond) initiation of biomolecular dynamics. Peptide models and non-perturbing site-specific IR probes were utilized to study mechanisms of folding and aggregation 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. Furthermore, side-chain interactions were probed to get insights into fibril formation. In pH-jump studies, folding of a pH-sensitive peptide was induced photochemically to investigate coiled-coil formation beyond the diffusion limit. Both perturbation techniques were applied to poly-L-glutamic acid (PGA), a peptide whose helicity is regulated by the protonation degree of its carboxyl side-chains. T-jump and pH-jump methods have been applied to PGA before, however never in combination with each other. We designed our experiments so that the same final PGA conformation was adopted after the pH-jump (folding) respectively T-jump (unfolding). We quantified the increase/decrease in helicity and could independently observe similar kinetics for helix folding/unfolding irrespective of which perturbation technique was used.

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### P-076. Structure-function relationship of RHO kinase I

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The serine/threonine Rho-associated protein kinases (RHO kinases I/II), which are key determinants in many fundamental cellular functions, serve as distinguished therapeutic targets in the treatment of a wide variety of diseases, particularly cardiovascular diseases. In contrast to the terminal globular kinase domain and the PH-C1 tandem little is known about the sequence -structure-function relationship of the central amphipathic α-helical segment of the RHO kinase proteins. Thus, a major aim of this study was to elucidate the structural basis of the entire segment of RHO-kinase I by using multiple biophysical platforms, including multi-angle light scattering (MALS), analytical ultracentrifugation (AUC), small-angle X-ray scattering (SAXS), negative stain electron microscopy (EM), and X-ray crystallography. The obtained results together with structural reconstitution of full-length RHO kinase I revealed that a highly elongated coiled-coil structure may switch between a dimeric and a tetrameric state. These and unexpected data obtained from measurements of enzymatic activity of full-length RHO-kinase I about the role of RHOA as a RHO kinase activator will be presented and discussed.

Keywords: Rho kinase, ROCK, Serine/threonine kinase, Coiled-coil, Structure function relationship

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### P-077. Micro- and nanostructured surface architectures for label-free spectroscopic and microscopic protein sensing

Julia Flesch[1], Christian Fabri[1], Maximilian Bettenhausen[2], Marcin Kazmierczak[3], Subhajit Guha[3], Wolfgang Klesse[3], Giovanni Capellini[3], Thomas Schroeder[3], Bernd Witzigmann[2], Changjiang You[1], Jacob Piehler[1]

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Most of the complex functions of proteins in cells result from their ability to specifically recognize other biomolecules in a highly regulated manner and to propagate this information via structural reorganization. While screening for drugs that efficiently block binding sites of proteins is well established, probing conformational changes involved in downstream propagation remains challenging. We here aimed to develop surface architectures that enable efficient interrogation of protein interaction and conformational organization by label-free surface-enhanced spectroscopy techniques. To ensure structural integrity and full functionality of immobilized proteins, we developed surface biofunctionalization for site-specific protein capturing into micro- and nanostructured sensor surfaces both in vitro and in live cells. Two label-free sensing approaches were explored: Silicon micropillar (SiMP) arrays combined with orthogonal surface chemistry were used for high density in situ protein capturing from cells cultivated on-chip. Protein interrogation was carried out by FTIR spectroscopy which enables optimal interrogation of IR-active secondary and tertiary protein structures by IR field enhancement via the SiMP sidewalls. As a second approach gold nanoparticles (AuNP) immobilized onto a glass surface were employed for localized surface plasmon resonance (LSPR) detection. Surface functionalization of immobilized AuNP with tris-(nitrilotiacetic acid) or HaloTag-Ligand yielded efficient site-specific capturing of His or HaloTag fusion proteins that allowed real-time monitoring of protein interaction by LSPR. By micropatterning of AuNP for LSPR-based reflection microscopy, we aim to achieve label-free LSPR imaging of interactions with membrane proteins of living cells. Moreover, integrating AuNP on SiMPs will allow highly sensitive interrogation of protein conformations by surface-enhanced infrared absorption spectroscopy (SEIRAS).

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### P-078. Integrated NMR, fluorescence and MD benchmark study of protein mechanics and hydrodynamics

Jakub Kubiak[1], Christina Möller[2,3], Oliver Schillinger[2,4], Ralf Kühnemuth[1], Dennis Della Corte[2], Gunnar F. Schröder[2,5], Dieter Willbold[2,3], Birgit Strodel[2,4], Claus A. M. Seidel[1], Philipp Neudecker[2,3]

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Understanding the function of a protein requires not only knowledge of its tertiary structure but also an understanding of its conformational dynamics. Nuclear magnetic resonance (NMR) spectroscopy, polarization resolved fluorescence spectroscopy and molecular dynamics (MD) simulations are powerful methods to provide detailed insight into protein dynamics on multiple timescales. Using these techniques, we present an integrated approach to study the dynamics of the autophagy-related protein GABARAP in its cytosolic form on the pico- to nanosecond timescale and its rotational and translational diffusion for protein concentrations spanning nine orders of magnitude. We compare the dynamics of GABARAP as monitored by $^{15}$N spin relaxation of the backbone amide groups, fluorescence anisotropy decays and fluorescence correlation spectroscopy of side chains labeled with BODIPY FL, and molecular movies of the protein from MD simulations. The data from all these techniques are analyzed and interpreted within a joint theoretical description of depolarization and diffusion to demonstrate the conceptual similarities. We provide the software package MOPS[2] for calculating order parameters and corresponding relaxation times from MD trajectories for comparison with the values determined experimentally by NMR and fluorescence spectroscopy, and examine the accuracy of our different methods by benchmarking them against each other. For the global rotational diffusion correlation time of GABARAP we find a consistent value of 8.0 ns ± 0.3 ns in sub-micromolar aqueous solution at 25°C, which corresponds to a hydrodynamic radius of 20.8 Å ± 0.3 Å, including a hydration shell of ≈ 3 Å. We propose a method to identify potential hinges for large-scale, functionally relevant intra-domain motions, such as residues 27/28 at the interface between the two sub-domains of GABARAP.

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### P-079. Collective force generation by elastically coupled molecular motors

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Many active cellular processes such as organelle transport, spindle organization, and beating of flagella and cilia are driven by molecular motors such as kinesin, dynein and myosin. These motor proteins act collectively to generate large forces, examples are the large-scale organization of the mitotic spindle by kinesin and dynein, and action of myosin during actomyosin contraction. The collective force generation of different types of motors can be additive or sub-additive, depending on the motor type. Both experimentally and theoretically, however, the underlying mechanisms remain controversial. Here we address this question by introducing a theoretical model for cargo transport by elastically coupled molecular motors. We elucidate the collective force generation of different types of motors and show that the vatiability of these collective processes is mainly determined by the difference in the unbinding behavior of single motors. We find that motors with a high stall force cooperate more poorly than weak motors due to an increased probability of strain-induced unbinding from the filament. Forces generated by weak motors, on the other hand, can even increase super-additively with increasing motor number for motors with low force-dependent unbinding rates or for fast and weak motors. We compare our theoretical results with experimental data on kinesin-1, which shows that the sub-additive force generation arises from strain-induced unbinding and not from unequal force sharing between the individual motors.

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### P-080. Multi-species diffusion studies in membranes utilizing scanning FCS and super-resolution microscopy

Maria Loidolt-Krüger[1], Fabian Jolmes[1], Mariano Gonzalez Pisfil[1,2], Marcelle König[1], Benedikt Krämer[1], Paja Reisch[1], Rhys Dowler[1], Felix Koberling[1], Matthias Patting[1], Uwe Ortmann[2], Andreas Herrmann[2], Rainer Erdmann[2]

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At present, fluorescence fluctuations of single molecules can be observed thanks to techniques such as Fluorescence Correlation Spectroscopy (FCS). Over the last decade, this method has allowed elucidating complex cellular processes.

Although being a very powerful tool, FCS has significant drawbacks for slower moving molecules like fluorophores diffusing in model or cell membranes. Photobleaching is one of the main issues. Furthermore, in order to average over a sufficient number of independent events the optimal measurement time for an FCS measurement is increased for slower moving species which in turn increases the chances of introducing artifacts (e.g., drift, or sample movement).

Scanning FCS (sFCS) was developed to counteract these issues. In this scenario, photobleaching is decreased all while the statistical accuracy is increased. In sFCS an increased number of molecules can be observed in a given time period compared to conventional FCS due to the scanning behavior. As an added advantage, the scanning process allows determining the volume without prior calibration.

As we use the confocal time-resolved fluorescence microscope MicroTime 200 STED, we also have access to the fluorescence lifetime information. In our case, multi species STED imaging and STED FCS is performed with a single STED laser, where the labels can be discriminated by applying a unique pattern matching analysis method.

In contrast to STED FCS, STED scanning FCS will allow to obtain data about hindered diffusion of fluorophores in membranes with observation spots freely tunable to smaller than 50 nm, overcoming not only averaging issues along long transit paths, but also reducing photobleaching, measurement times and improving statistics. Our pulsed interleaved excitation (PIE) illumination scheme allows to check online in a straightforward way whether the STED laser has an influence on the investigated dynamics in STED-FCS.

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### P-081. Dynamic and structural properties of polyglutamine

Michael Schleeger[1], Peter Enke[1], Thomas Kiefhaber[1]
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At least ten diseases are known, which are caused by proteins with expanded polyglutamine (polyQ) sequences eventually forming fibrillar deposits named amyloids. We tested, whether the propensity to form amyloids is due to distinct dynamic or structural properties of the monomeric state of polyQ chains. Using triplet-triplet energy transfer (TTET) and time-resolved fluorescence resonance energy transfer (trFRET) we were able to characterize the kinetics of intrachain loop formation, the intrachain diffusion coefficient for relative motions between donor and acceptor and the end-to-end distance distribution of polyQ chains of different length. The results were compared to the properties of the homopolymeric model chains poly-(glycine-serine) and poly-serine and of fragments from IDPs, which do not form amyloids. The results show that the end-to-end distance distribution of polyQ is similar to those of soluble chains of similar length but polyQ contains a significant fraction (14%) of pre-formed loop structures. Intrachain loop formation in polyQ is slower than in all other investigated chains due to slowed down internal chain motions, which may be caused by H-bonding between side-chain and backbone groups. These slow chain dynamics in combination with the high fraction of pre-formed loop structures may promote intermolecular interactions during self-assembly into aggregates.

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### P-082. Comprehensive biophysical assays: From single channel electrophysiology to overall cell behavior

Conrad Weichbrodt[1], Ilka Rinke-Weiss[1], Nadine Becker[1], Krisztina Juhasz[1], Ekaterina Zaitseva[2], Gerhard Baaken[2], Alison Obergrussberger[1], Matthias Beckler[1], Michael George[1], Sonja Stoelze-Feix[1], Andrea Brüggemann[1], Niels Fertig[1]

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Nanion is a leading provider of automated patch clamp instrumentation with throughput capabilities ranging from 1, 8 and up to 768 cells in parallel, combining bio- and microtechnology for high quality products serving the life sciences and drug discovery. Since 2004, Nanion has successfully introduced four automated patch clamp platforms, the Port-a-Patch, Patchliner, the SyncroPatch 96 and the SyncroPatch 384/768PE, which are highly appreciated tools in academic and industrial settings. Nanion also provides devices for cardiotoxicity screening, parallel bilayer recordings and membrane transporter protein recordings. We are your one-stop-shop for ion channel research, drug discovery and safety screening.

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### P-083. Connexin 43 as a model to mimic cell-to-cell communication in minimal cell compartments (MCCs)

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Intercellular communication is an important process in multicellular organisms. Gap junctions are specialized membrane regions containing hundreds of intercellular communication channels that allow the passage of molecules such as ions, metabolites, nucleotides and small peptides. A gap junction is a channel formed by end-to-end docking of two hemichannels, also referred as connexons, each composed of six connexin subunits. Connexin 43 (Cx43) is the major isoform of the connexin family. The aim of this study is to mimic cell-to-cell communication through the gap junction system by using a bottom up approach, for that Connexin 43 protein and Giant Unilamellar Vesicles (GUVs) will be used as a model system. The project has been designed in three stages: 1) use of molecular tools to isolate, amplify and sequence Cx43 DNA, 2) production Cx43 protein by using the cell free expression system (PURExpress®), and 3) characterization of the protein activity at the hemichannel and gap junction level using the Black Lipid Membrane technique. We have successfully isolated and amplified the DNA for the production of Cx43 protein. Furthermore, we will reconstitute Cx43 in GUVs and work on the insertion of the protein into lipid bilayers by using the BLM system to electrophysiologically characterize the protein at the single channel level as well as to mimic cell-to-cell communication between linked Cx43 functionalized GUVs.

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### P-084. Structure and mechanics of the membrane-bound intermediate filaments

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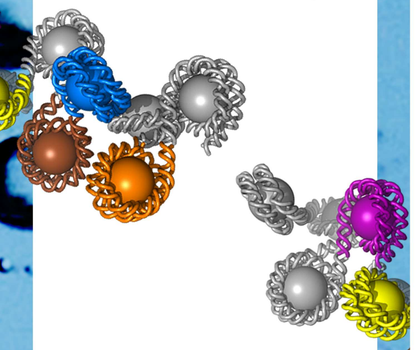
In eukaryotic cells, the shape and their mechanical properties are determined by the cytoskeleton, a highly intertwined network which includes three types of biopolymers: actin filaments, microtubules and intermediate filaments. While actin filaments and microtubules are highly conserved, intermediate filaments share a common secondary structure that is organism and tissue specific. Importantly, intermediate filaments are considered to be the main determinants of the cell stiffness and strength. The reason is that they can withstand much larger deformations - in contrast to the other both cytoskeletal components. Thus, they are believed to dominate the mechanical response of the cells at higher strains, even though they tend to be softer at low strain. Therefore, the intermediate filament organization at the plasma membrane and their influence on the mechanical properties of the cells are important to investigate, especially under higher strains.
In our in vitro model system, to mimic the situation at the plasma membrane, biotin-labeled intermediate filaments are attached to biotin-decorated lipid bilayer via neutravidin. It has a higher affinity to biotin than streptavidin or avidin and its neutral charge prevents nonspecific bindings. The lipid bilayers are preliminarily prepared on silicon wafer supports. Confocal laser scanning microscopy is used to monitor the architecture and the organization of intermediate filaments and the surface topography as well as the elastic properties are imaged by means of atomic force microscopy.

## Notes





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### P-085. Synthetic cells, a reductionists approach: Reconstitution of an ATP synthase in giant unilamellar vesicles by droplet based microfluidics

Kristina Kramer[1], Hannes Witt[1,2], Miriam Schwamborn[1], Claudia Steinem[1,2]

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The understanding of the living matter is one of the most important and challenging targets in modern research. The formation of an interconnected system of artificial multifunctional minimal cell compartments (MCCs), which have essential biological components implemented to allow for collective behaviour of the artificial tissue is a promising strategy to study complex processes in living systems. The best suited model system to reach a cell-like system are giant unilamellar vesicles (GUVs), equipped with divers proteins. One necessary requisite towards artificial living material is the reconstitution of a protein that can act as a renewable energy source for the system.

To generate energy in the form of ATP through electrochemical gradients, the FOF1 ATP synthase is used and for functional insertion of this protein into a GUV, physiological conditions have to be retained in the environment. For this intended purpose modular engineering approaches relying on droplet based microfluidic systems to generate (droplet stabilized) GUVs and to inject proteins in a physiological and gentle manner are appropriate. An alternative way to remain a physiological environment of the liposomes is the electrostatically mediated fusion of smaller proteoliposomes with GUVs preformed via electroformation. To monitor and study the activity of the ATP synthase in GUVs and MCCs, pH sensitive assays with fluorescent dyes or the ATP sensitive and very common Luciferin-Luciferase assay can be used.

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### P-086. Investing the organization of minimal actin cortices and their impact on pore-spanning lipid membrane tension by means of AFM - CLSM correlation

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During a life time, cells have to modify and adapt their membrane shape, mechanical properties and driving forces several times. The cytoskeleton structures enable this adaption. Filamentous actin (F-actin) is one of the main proteins in this highly regulated biological network, which forms ordered structures like the actin cortex. A connection between the actin cortex and the plasma membrane is necessary to convert mechanical forces between both structures. With a direct connection, F-actin is able to vary membrane tension for different plasma membrane related processes and to respond on external forces. In vivo F-actin binds by means of actin binding proteins like ezrin, radixin and moesin (ERM-family) and the membrane receptor-lipid phosphatidylinositol-4,5-bisphosphate (Ptd(4,5)Ins).

In this work an artificial F-actin network is electrostaticly recruited to pore-spanning lipid bilayers (PSLBs). Besides the investigation of network specific parameters like mesh size and pinning point density via confocal laser scanning microscopy (CLSM) the mechanical membrane properties are analyzed by means of atomic force microscopy (AFM). A direct correlation of both methods displays the local differences in F-actin organization, which can be directly compared with changes in lateral membrane organization and tension. Utilizing this approach, the impact of F-actin on the membrane tension can be examined without disturbing components like actin binding proteins. Furthermore, this approach enables the possibility of stepwise addition of these components for further experiments.

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## Poster Presentations

### P-087. Cytoskeletal and membrane reorganization during NET formation

Elsa Neubert[1], Daniel Meyer[2,3], Anja Kwaczala-Tessmann[1], Susanne Senger-Sander[1], Gökhan Günay[1,2], Julia Grandke[1], Michael P. Schön[1,4], Luise Erpenbeck[1], Sebastian Kruss[2,3]

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Neutrophils can catch and kill pathogens by expelling a fibril network made from their own DNA (Neutrophil Extracellular Traps, NETs). During this process, cells rearrange their contents profoundly. Within a few hours, the nuclear chromatin expands until it fills the whole cell. Finally, the decondensed chromatin is released through the cell membrane into the extracellular space, ultimately leaving the neutrophils to die. A detailed analysis of the fine-tuning of these processes, however, would support a deeper understanding of this fundamental immunological mechanism.

This study focuses on the involvement of cytoskeletal and membrane rearrangement during the formation of NETs. After activation with phorbol 12-myristate 13-acetate (PMA), particular cytoskeletal components as well as the cell membrane of human neutrophils were stained and observed time-resolved by confocal laser scanning microscopy (CLSM), reflection interference contrast microscopy (RICM) and atomic force microscopy (AFM). Additionally, the impact of cytoskeletal inhibitors (cytochalasin D, latrunculin A, jasplakinolide and Y-27632) on NETosis was evaluated.

Our results show, that major components of the cytoskeleton such as microtubules and actin filaments, degrade during the time-course of NETosis. Additionally, the impact of cytoskeletal inhibitors of NETosis massively decreases over time, supporting the loss of cytoskeletal function. This degradation of the cytoskeleton is further accompanied by cell rounding and significant softening. Furthermore, by precise analysis of membrane dynamics, we found a biomechanically predetermined breaking point of the cell membrane before NET release.

Based on these findings, we hypothesize that the loss of cytoskeletal integrity together with the alterations in the cell”s shape and stiffness are mechanical requirements for the final rupture of the cytoplasmic membrane and release of DNA.

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### P-088. Effect of substrate elasticity on neutrophil extracellular trap formation

Gökhan Günay[1], Sebastian Kruss[1,2], Micheal Schön[1,2], Luise Erpenbeck[1,2,3], Florian Rehfeldt[1,2,3], Daniel Meyer [1,2,3,4], Elsa Neubert[1,2,3], Galina Kudryasheva[1,2,3]

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Neutrophils are the most abundant phagocytes and contribute to both innate and adaptive immunity through phagocytosis, secreting anti-microbials and degranulation. They are also able to perform a new type of cell death called neutrophil extracellular trap formation (NETosis). It is a regulated form of neutrophil cell death in which neutrophils release their decondensed chromatin, neutrophil extracellular traps (NETs), decorated with cytosolic and granule proteins. They play a role in inflammation and bacterial infections, however they have been also linked to non-infectious diseases such as; systemiclupus erythematosus (SLE), rheumatoid arthritis (RA), vasculitis, diabetes, atherosclerosis and cancer. Increased NETosis or decreased NET clearance likely increases the risk of autoreactivity to NET components. Even though there are several studies on characterizing NETosis and its functionality, biological significance of NETosis is still being explored, to our knowledge there are no studies investigating the effect of stiffness on NETosis. Considering that stiffness can have influence on cellular functions we investigated the effect of stiffness on NETosis. Freshly isolated human neutrophils were seeded on polyacrylamide (paa) gels with different substrate elasticity (1kPa, 2kPa, 4kPa, 8kPa, 16kPa, 20kPa, 30kPa, 60kPa, 128kPa) coated either with collagen I or fibrinogen. The cells were stimulated to perform NETosis either with phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS). We found that under PMA stimulation the NETosis did not depend on substrate elasticity. However, LPS stimulation caused significantly higher NETosis rate on stiffer gels. Change in NETosis rate with stiffness could provide new insights on their functionality on diseases such as atherosclerosis in which there is an increment in arterial stiffness or in Alzheimer”s disease in which the brain stiffness is decreased.

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### P-089. Spontaneous shear flow in confined cellular nematics.

Carles Blanch-Mercader[1]

[1]Universite de Geneve, Biochemistry, Genève, Switzerland

In the last decades, a great deal of attention has been focused on the behavior of epithelial monolayers, as a model system to study morphogenesis, tissue repairing or cancer invasion. Here, I will present recent experimental findings on a novel mode of collective cell migration found in ensembles of spindle-shaped cells that are plated in adhesive stripes in vitro [1]. On wide stripes, the cells self-organize in a nematic phase with a director at a well-defined angle with the stripe”s direction, and develop antiparallel cell flows close to the stripe”s edges. However, on stripes narrower than a critical width, the cells perfectly align with the stripe”s direction and the net flow vanishes. Interestingly, such antiparallel streams have been reported in vivo in embryonic development and cancer. I will present a hydrodynamic active gel theory that describes faithfully our experimental findings and identifies the transition between the non-flowing phase oriented along the stripe and the tilted phase exhibiting shear flow as a transition driven by the actively generated forces among cells. Our results suggest that antiparallel cell displacements that contribute to cell transport in vivo, may be a generic outcome of the confinement of active nematic gel properties of cellular assemblies.

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### P-090. A model system for cellular adhesion based on microstructured substrates

Jonathan F. E. Bodenschatz[1], Andreas Janshoff[1]

[1]University of Göttingen, Biophysical Chemistry, Göttingen, Germany

The adhesion of cells to an extracellular matrix is primarily mediated by clusters of adhesive proteins called focal adhesions. In order to study the underlying principles of the formation of such clusters we use giant unilamellar vesicles (GUVs) which have been functionalized with adhesive molecules. These vesicles adhere to functionalized microstructured polydimethylsiloxane (PDMS) substrates. The substrates can be rapidly changed and adjusted by use of a 3D-printer to allow for an optimal substrate form as well as quick and rapid substrate production. Due to the nature of these substrates, measurement of the adhesion has proven difficult. Currently we use reflection interference contrast microscopy, which provides detailed information about distance between the GUVs and the substrate. By changing the topography of these substrates we aim to apply different forces onto these clusters.

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### P-091. Is ATP Hydrolysis the Power Stroke of ABC Transporters?


Hendrik Göddeke[1], Marten Prieß[1], Gerrit Groenhof[2], Lars Schäfer[1]

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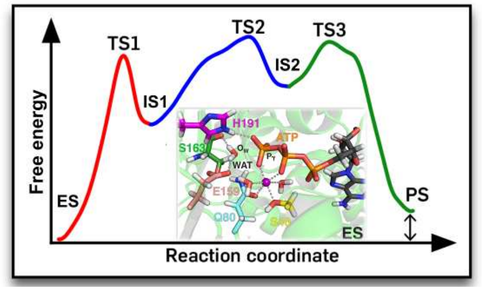
Hydrolysis of nucleotide triphosphate (NTP) plays a key role in the function of many proteins. However, the chemistry of the catalytic reaction in terms of an atomic-level understanding often remains elusive. We studied the molecular mechanism of adenosine triphosphate (ATP) hydrolysis in the ATP-binding cassette (ABC) transporter BtuCD-F. Free energy profiles obtained from hybrid quantum mechanical/molecular mechanical (QM/MM) molecular dynamics (MD) simulations reveal that the hydrolysis reaction proceeds in three steps. The first step is the nucleophilic attack of an activated lytic water molecule at the ATP-phosphate to yield ADP + $HPO_4^{2-}$ as intermediate product. A glutamate residue that is located very close to the phosphate transiently accepts a proton and thus acts as catalytic base. In the second and third step, this proton is transferred back to $HPO_4^{2-}$, yielding ADP + $H_2PO_4^-$ as final products. The rate estimated from the computed free energy barriers is in very good agreement with experiments. The overall free energy change associated with the reaction is positive, suggesting that ATP hydrolysis itself does not provide the power stroke for substrate transport in BtuCD-F. The proposed mechanism is likely relevant for all ABC transporters and might have implications also for other NTPases, thus representing a key step towards understanding chemo-mechanical energy conversion in NTP-driven molecular machines

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### P-092. The Multiple Origins of the Hydrophobicity of Fluorinated Apolar Amino Acids

Joao Robalo[1], Ana Vila Verde[1]

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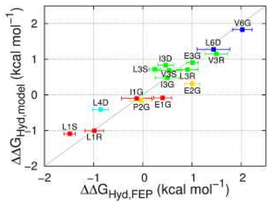
Fluorinated amino acids – e.g. amino acids where the side chain methyl groups are substituted by mono, di or trifluoromethyl groups – have been shown to affect the thermal stability of proteins. Key to this effect is the change in the hydrophobicity of fluorinated amino acids versus their non-fluorinated counterparts. This change is interpreted on the basis of contributions of both surface area and polarity. However, the experimental quantification of either contribution is yet insufficient to either predict or clarify the impact of fluorination on proteins. In this work, we investigate the mechanism underlying the interaction between fluorinated amino acids and water by constructing atomistic force fields of fluorinated apolar amino acids for molecular dynamics simulations and calculating their hydration free energy with free energy perturbation simulations. We find that fluorination can render amino acids either more hydrophobic or more hydrophilic, with only a small correlation with the degree of fluorination. Although a surface area contribution to the hydration free energy changes can be easily quantified via Lennard-Jones interactions, electrostatics requires a decomposition into several parameters, namely different types of hydrogen bonds and individual electrostatic interactions between the amino acid side chains and adjacent water molecules. Collecting these various contributions into a linear model, we can qualitatively reproduce the trend in fluorine-induced changes in hydration free energy and identify the underlying mechanisms that allow fluorine, the most electronegative element, to make a molecule more hydrophobic.

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### P-093. Recognition and Specificity in the Initial Steps of the Base Excision Repair Mechanism

Petra Imhof[2], Natal Kanaan Izquierdo[1], Hossein Batebi[2,3]

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The Base excision repair (BER) system is a machinery of enzymes, recognising, removing, and correcting mispairs in the DNA. In the first step of the base excision repair system glycosylases recognise a damaged or mispaired base and remove it via glycosidic C1'-N1 bond hydrolysis. Apurinic/apyrimidinic endonuclease (APE) then cleaves the DNA backbone at the abasic site so as to allow subsequent insertion of a new, correct nucleotide by polymerase βa and ultimately sealing of the backbone by a ligase enzyme.

A crucial step in the base recognition and excision of mispared thymine (or damaged) bases by the BER enzyme human thymine DNA glycosylase (TDG) is the extrusion of the substrate base of the DNA helix and its "flip" into the active ste of the enzyme.

The intrinsic conformational dynamics of mispaired DNA, exhibiting a partially-opened, partially flipped state [1], is exploited by the enzyme, stabilising this state over a closed state in the prrotein-DNA complex [2]. Further discrimination is achieved by the substrate base being better acommodated in the active site than non-cognate bases [2].

The glycosidic bond scission in the enzymatic complex is via a step-wise dissociative mechanism and largely facilitated by a proton transfer to the leaving base that is unlikely for intact cytosine bases. The chemical step can thus be understood as the last of several instances to protect intact DNA from base excision [3].

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### P-094. Analysis of Networks in Androgen and Glococorticoid receptors in complex with DNA

Senta Volkenandt[1], Mahdi Bagherpoor Helabad[1], Petra Imhof[1]
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We compared the interaction of the glucocorticoid (GR) and the androgen (AR) receptor in complex with a direct (DR) or an inverted (IR) repeat response element with the help of MD simulations. For the comparison we mainly used the first and last 100ns of the 500ns simulations to calculate different metrics for communication. We showed that H-bonds and correlations are important metrics to consider. Community analysis revealed that the complexes consist of five bigger core communities and several smaller communities. The main communities are all connected to each other and thus imply that the two monomers, the dimerisation region and the two hexamers of the DNA can act as allosteric ligands for one another. For communication between the hexamers and the diagonal monomer the spacer region is an important mediator.

Besides similarities among the AR systems and the GR systems, we also found some for AR-DR and GR-IR, and AR-IR and GR-DR, suggesting a higher specificity of the former because GR does not bind to DR. Monomer A of GR-DR is tilted slightly around the DNA and shows in the end much higher van der Waals and H-bond interaction. Mainly responsible for that is a strong H-bond between HSP472 and the DNA that cannot be found for the other systems. The establishment of that strong H-bond follows the distance of the zinc ions of the second zinc fingers. This is made possible after the H-bond between HSP472 in B and DNA breaks.

From time lagged generalised correlation analysis we learned that the whole complexes, especially the monomers, are constantly in motion as the residues always influence each other in their movement.

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### P-095. Reaction path prediction in proton transfer systems

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The translocation of protons from one side of a biological membrane to the other is an exceptionally important process in nature. To receive a comprehensive picture of the proton translocation through individual proton transfer channels the well orchestrated interplay of various degrees of freedom, acting on different time and length scales, needs to be elucidated. Transition Networks translate complex reactions into networks of simpler transitions, thereby allowing bias-free investigations using path optimization methods. A challenging aspect in calculating Transition Networks is the exponential increase of stationary points on the energy surface with increasing system size, rendering a direct inclusion of all degrees of freedom, involved in the proton translocation process, infeasible. Hence, several re-calculations of the Transition Networks need to be performed with varying configurations of the unsampled degrees of freedom to gain a comprehensive understanding of this exceptionally important process. Here, we present a method, which determines coarse-grained Transition Networks, using simple graph theoretical algorithms, for different configurations of the unsampled degrees of freedom, starting from an initial Transition Network calculation, thereby reducing the re-calculation costs by up to 50 %, while maintaining important network properties, e.g. the minimax barrier of the proton translocation process.

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## Poster Presentations

### P-096. Long dynamic simulations of deinococcus radiodurans bacterial phytochrome

Giovanni Battocchio[1], Maria Andrea Mroginski[1], Tillmann Utesch[1]

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Phytochromes are bimodal photoswitches found in plants, bacteria, cyanobacteria, algae and fungi. They can reversibly photoconvert between two states, the red-light absorbing state (Pr) and the far-red-light absorbing state (Pfr), upon illumination and thermal relaxation. By means of long molecular dynamics (MD) simulations we investigated the dynamical properties and correlated motions of Deinococcus Radiodurans bacterial phytochrome in the Pr state. Conventional classical Molecular Dynamic (cMD) was complemented by accelerated Molecular Dynamic (aMD) in order to enhance the sampling of the conformational space. Furthermore, reproducibility of the resulting trajectories was evaluated by comparing different force fields (Amber and Charmm). The dynamic differences between monomer and dimer in the long time simulations are confronted and discussed.

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### P-097. Modulation of TRPV1 heat activation mechanism by the lipid membrane.

Anna Bochicchio[1], Rainer A. Böckmann[1]

[1]Friedrich-Alexander Univesität Erlangen-Nürnberg, Computational Biology, Erlangen, Germany

Temperature activated TRP channels are unique among ion channels in that they can be activated by changes in temperature alone. In particular, several members of the TRPV family respond to different ranges of high temperatures and contribute to the physiological sensation of temperature changes in mammals and other organisms. The biophysical response of TRPV1 to temperature change is a large increase in current, produced both by an increase in the channel open probability and in the single-channel conductance, with the open probability showing the larger temperature dependence. The mechanism by which these channels accomplish the conversion of the absorbed heat into a conformational change that eventually triggers channel opening remains largely unknown. In contrast with the voltage sensor of voltage-gated channels, a “temperature sensor” has not been found and it has been suggested that temperature might affect several regions of the channel protein at once.

Here, by using coarse-grained and atomistic molecular dynamics simulations, we are investigating how the membrane properties (e.g. the membrane thickness, plasticity, or the lateral pressure profile) influence the TRPV1 sensing mechanism and its closed-to-open conformational transitions.

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### P-098. Dynamic hydrogen-bond networks of channelrhodopsin variants

Michail Lazaratos[1], Malte Siemers[1], Ana-Nicoleta Bondar[1]

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Channelrhodopsins are membrane-embedded proteins that couple photo-isomerization of the retinal chromophore with proton transfers and passive flow of cations. A key open question is how protons are transferred across long distances in the polar environment of channelrhodopsin variants with different amino acid sequences. To address this question, we perform extended all-atom simulations of channelrhodopsin variants embedded in hydrated lipid membranes, and implement algorithms for the efficient analysis of large data sets on dynamic hydrogen-bond networks. The analyses indicate that the interior of channelrhodopsin hosts extensive networks of protein/water hydrogen-bond networks that rapidly respond to perturbations such as mutation or changes in the protonation state.

**Acknowledgements**

Financial support was provided in part by the DFG Collaborative Research Center SFB 1078 Project C4 (to A.-N.B.) and by the Freie Universität Berlin within the Excellence Initiative of the German Research Foundation. An allocation of computing time was provided by the HLRN, the North-German Supercomputing Alliance (bec00063).

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### P-099. Computational ssimulations of zinc binding to the dimeric human voltage-gated proton channel

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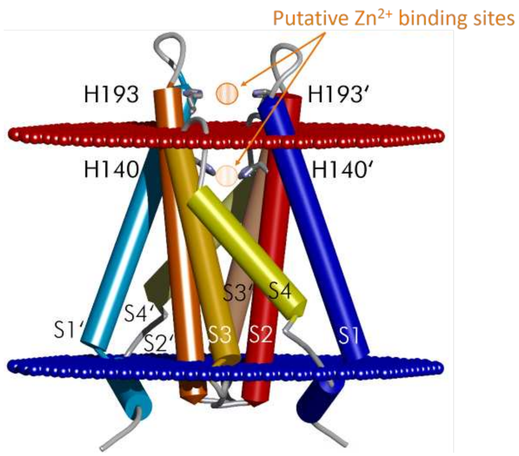
$H_v1$ voltage-gated proton channels are proton-specific ion channels with unique properties. For example, they are massively expressed in human sperm where they are necessary for maturation and motility, hence essential for conception. The channels are strongly inhibited by $Zn^{2+}$. Experimental studies revealed that histidine residues are essential for $Zn^{2+}$ binding. However, the two accessible histidine residues (H140 and H193) in the monomeric channel are too far apart to coordinate simultaneously one $Zn^{2+}$. It was thus suggested that two $Zn^{2+}$ binding sites can be formed between pairs of equivalent histidine residues at the interface of a $H_v1$ homodimer. The consecutive experimental measurements were also in agreement with this hypothesis.

To test this hypothesis and understand the determinants of $Zn^{2+}$ binding at the molecular level, we used a computational approach: molecular modeling was used to build structural models of the human $H_v1$ ($hH_v1$) channel, and molecular docking was used to generate putative models of the dimer. The appropriate docking models were submitted to molecular dynamics (MD) simulations in order to simulate and analyze the binding of $Zn^{2+}$ to $hH_v1$ in a realistic (physiological) environment at the molecular level.

The simulations show that the $hH_v1$ channels can form homodimers that present an adequate interface with two zinc binding sites each involving a pair of equivalent histidine residues from each monomer. The MD simulations reveal that the zinc binding sites are tetrahedral. In addition to the histidine residues, the binding sites are composed of either two acidic residues (Asp or Glu), or one acidic residue and one water molecule. Essentially, the glutamate residues E192 play an essential role in zinc binding.

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## Poster Presentations

### P-100. Reliable state identification and state transition detection of fluorescence intensity-based smFRET data

Richard Börner[1], Melodie C.A.S. Hadzic[1], Danny Kowerko[2], Sebastian L.B. König[1], Marc Ritter[3], Roland K.O. Sigel[1]

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[2]Chemnitz University of Technology, Department of Computer Science, Chemnitz, Germany
[3]Mittweida University of Applied Sciences, Department of Applied Computer and Biosciences, Mittweida, Germany

Single-molecule Förster resonance energy transfer is a powerful technique to probe biomolecular structure and dynamics. A popular implementation of smFRET consists in recording fluorescence intensity-time traces of surface-immobilized, chromophore-tagged molecules. This approach generates large and complex datasets, and several methods for video processing and analysis have been reported. As these algorithms often address similar aspects in video analysis, there is a growing need for standardized comparison. We developed a MATLAB-based Multifunctional Analysis Software for Handling smFRET data (MASH-FRET, https://github.com/RNA-FRETools/MASH-FRET) that allows for the analysis and simulation of camera-based smFRET videos (1-3). Here, we use MASH-FRET to simulate standardized data sets, suitable for benchmarking video processing algorithms (2). Specifically, we generate thermodynamic and kinetic models that describe with statistical rigor the behavior of FRET trajectories recorded from surface-tethered biomolecules, i.e., the number of FRET states, the corresponding FRET values and the kinetic rates at which they interconvert taking heterogeneous FRET and intensity broadening into account. We survey how video test sets should be designed to evaluate currently available data analysis strategies in camera-based sm fluorescence experiments. Further, we provide an account on current strategies (HaMMy, ebFRET, vbFRET, STaSI, CPA, references provided in 3) to achieve model selection, including state identification and state transition detection, on smFRET trajectories as well as quantitative assessment of their performances (3). We evaluate the performance of each algorithm with respect to accuracy, reproducibility and computing time, which yields a referential benchmark of each algorithms' efficiency according to various data qualities and provide a best practice strategy (3).

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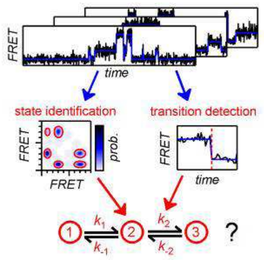


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### P-101. Probing fosfomycin permeation across the E. coli outer membrane

Vinaya Kumar Golla[1], Eulàlia Sans Serramitjana[2], Karunakar Reddy Pothula[1], Lorraine Benier[2], Jayesh Arun Bafna[2], Mathias Winterhalter[2], Ulrich Kleinekathöfer[1]

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Fosfomycin is a phosphonic acid antibiotic used in the treatment of urinary tract infections. The “old” antibiotic fosfomycin regained importance by showing activity against multidrug-resistant Gram-negative bacteria and extended spectrum β-lactamase producing pathogens. Moreover, fosfomycin shows a broad spectrum bactericidal activity by an irreversible inhibition of the UDP-N-acetylglucosamine-3-enolpyruvyl transferase (MurA) enzyme, thus hinder the peptidoglycan biosynthesis of the bacterial cell wall. In order to reach its cytoplasmic target MurA, the translocation of fosfomycin into the bacterial cell from the external environment is of key importance. The presence of outer membrane channel forming proteins facilitate the transportation of nutrients, hydrophilic substances and certain classes of antibiotics. Hence, we studied the translocation of fosfomycin through proteins of the E. coli outer membrane using all-atom molecular dynamics simulations, single channel conductance and zero-current assay experiments. The combined computational and electrophysiological findings help in understanding the translocation of fosfomycin from the extracellular space to the inside of the bacterium.

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### P-102. An amphipathic lipid packing sensor motif in Piccolino makes the protein a possible candidate for a synaptic vesicle tether

Sonja A. Kirsch[1], Kaspar Gierke[1], Johann Helmut Brandstätter[1], Hanna Regus-Leidig[1], Rainer A. Böckmann[1]
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Vision comprises a process that takes place in the retina of the eye: the conversion of light into neural signals and their transduction to the brain. The incoming light is processed by the photoreceptor cells, followed by signal delivery through several layers of distinct cells to the optic nerve connecting the retina with the brain. At the photoreceptor ribbon synapse, a large proteinaceous organelle (the synaptic ribbon) supports sustained neurotransmitter release by tethering hundreds of synaptic vesicles at the active zone. However, the protein responsible for vesicle capturing at the synaptic ribbon is still unknown. Initial experiments suggest that Piccolino, a splice variant of the active zone protein Piccolo could be responsible for this task. Supporting this view, ultrastructural analysis of a Piccolino mutant rat revealed a loss of vesicle accumulation at the synaptic ribbon in rod photoreceptor terminals. Recently, a highly curvature-sensitive, amphipathic lipid packing sensing (ALPS) motif was detected in Piccolo. This helix characteristically rich in polar but poor in basic residues is also present in Piccolino. To investigate a possible role of Piccolino-ALPS in membrane binding and curvature sensing, molecular dynamics simulations were performed. Extensive 1µs long atomistic simulations of solvated ALPS showed a fast transition from water to the membrane-water interface. Alchemical simulations were conducted to obtain the relative binding free energy between wild type and mutant ALPS helices. A comparison of helix binding to membranes of different compositions showed that hydrophobic defects, also present in curved membranes, facilitate binding and stabilize a helical conformation. Membrane nanodiscs with the bound Piccolino-ALPS helix were observed to induce a positive membrane curvature.
These results hint to a possible role of Piccolino acting as a vesicle tether at the ribbon synapse via its ALPS motif.

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## Poster Presentations

### P-103. Understanding the structure and function of DcaP channel from Acinetobacter baumannii using the MD simulations

Jigneshkumar Dahyabhai Prajapati[1], Satya Prathyusha Bhamidimarri[2], Michael Zahn[3], Dirk Bumann[4], Bert van den Berg[3], Mathias Winterhalter[2], Ulrich Kleinekathöfer[1]

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DcaP is putative dicarboxylate specific channel, located in the outer membrane of the pathogen Acinetobacter baumannii. X-ray crystal structure reveals that DcaP is the first trimeric channel identified in Acinetobacter baumannii and could have a possible role in substrates and antibiotics permeation. To characterize the permeation properties of this channel, we have carried out the applied field MD simulations in presence of ions (KCl), substrates (phthalic acid, succinic acid) and β-lactam antibiotic (sulbactam). Additionally, the free energy calculation are also carried out using metadynamics simulations to identify the lowest energy permeation path along the 2D free energy surfaces and the most prominent residues required for the translocation. These simulations clearly suggest that DcaP channel is involved in the permeation of these solutes and results are complemented with electrophysiology experiments. Additionally, DacP channel forms an extended N-terminus domain which is presumably known to form the coiled-coil structure in the periplasmic space. As the N-terminus domain was not resolved in crystal structure, we have predicted the structure using the extensive modeling approaches. Moreover, the simulations and electrophysiology experiments suggest that the N-terminus might have a role in formation of stable trimer. Overall, we have built a great structure-function relationship for DcaP channel and it could help in designing next generation antibiotics having efficient permeability through this channel.

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## Poster Presentations

### P-104. The study of proton transfer in photosystem II

Krzysztof Buzar[1], Ana-Nicoleta Bondar[1]
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The proton transfer in Photosystem II is the crucial element of the proteins main function, which is the light driven water splitting reaction. Recent crystallographic studies of photosystem II [1-2] reveal interactions between the dimers of the protein suggesting dimer-dimer interactions having impact on the release of protons from the reaction centre. [3] To properly investigate the intrinsic influence of dimers on this process, simulations of dimer-dimer system is required. The system proposed for this study would consist of over 2.5 million atoms. To this aim we perform force-field parametrization of the manganese metal cluster of the oxygen evolution complex via quantum mechanical calculations and classical mechanical calculations of the dimer of dimers of  photosystem II protein.

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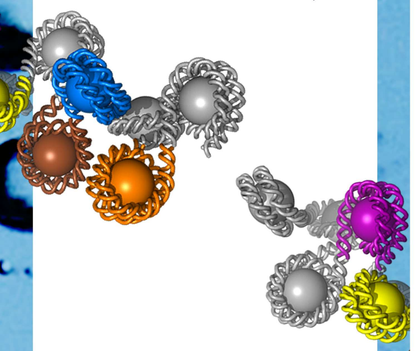



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## Poster Presentations

### P-105. Precise time super-resolution by event correlation microscopy

Qinghua Fang[1], Ying Zhao[1], Manfred Lindau[1]
[1]Max-Planck-Institute for Biophysical Chemistry, Laboratory for Nanoscale Cell Biology, Göttingen, Germany

The time resolution of fluorescence imaging is limited by the exposure time and readout time of the camera. Although many biological events are very fast, due to the weak fluorescence signal, the exposure times are typically much longer than many time scales of interest. It is often of particular interest to determine the time of a fluorescence change relative to a rapid event, such as an action potential or pulse stimulation, with high time resolution, higher than the limit given by the imaging frame durations. To break this time resolution limit, we developed an ECOM (Event COrrelation Microscopy) that take advantage of the fact that the intensity in a certain region of interest reported by an imaging frame depends on the time at which the intensity change occurred. The method depends on a simultaneous recording of the imaging data and a highly time resolved temporally related biological (physical) event such as a stimulation pulse or action potential. When multiple events occurring at random times during an exposure are averaged, the time correlation between the physical events and the fluorescence change can be determined with sub-frame resolution.

As a computational method for image analysis, without extra demand of sophisticated hardware and software, ECOM can theoretically achieve imaging temporal resolution down to any precision, limited only by the signal-to-noise ratio of the averaged imaging data. Analogous to super-resolution microscopy which beats the spatial resolution limit beyond pixel size, ECOM beats the time resolution limit beyond the imaging frame duration. The principle of ECOM and the ECOM precision limited by the signal-to-noise ratio (SNR) and other imaging factors will be reported.

The standard error of the ECOM detected time is:

$\sigma_t = SQRT(3/(2+\gamma)) * t_{frame}/SNR$, where $\gamma = t_{readout}/t_{frame}$ is the camera readout fraction of the acquired frames, $t_{frame}$ is the camera frame time, and SNR is the signal to noise ratio of the averaged trace.

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### P-106. Open boundary simulations of low-resolution membrane protein models

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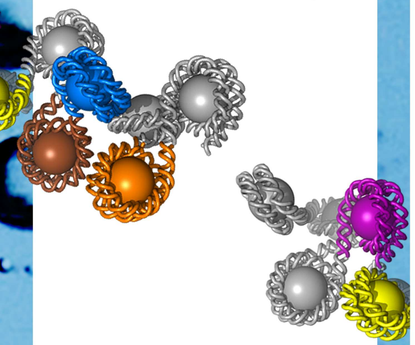
Multiscale molecular dynamics (MD) methods allow the coupling of different resolutions in the description of molecular systems, and are therefore particularly suited to exploit the intrinsic heterogeneity of biomolecular systems. In particular, the so-called Molecular Mechanics/Coarse-Grained approach (MM/CG), coupling in the same set-up atomistic and coarse-grained models, was shown to be an effective alternative to all-atom simulations when the absence of structural experimental data and the low sequence identity with templates limit the reliability of fully atomistic membrane proteins models. This is the case of G-protein-coupled receptors (GPCRs), which represent the most important family of targets for pharmaceutical intervention. Within the MM/CG scheme, the implementation of the so-called Hamiltonian Adaptive Resolution Scheme (H-AdResS) for the description of the solvent will lead to the simulation of a rigorous statistical ensemble – the grand-canonical one – suitable for the calculation of ligand binding free energies where traditional approaches may fail. Towards this goal, we applied for the first time H-AdResS to biomolecular systems, namely two atomistic proteins in dual-resolution solvent, proving its ability to reproduce structural and dynamical properties of both the protein and the solvent, as obtained from fully atomistic simulations. This approach has been subsequently integrated with the MM/CG scheme to simulate dual-resolution water in the presence of a membrane interface. This paves the way to the use of open-boundary simulations of membrane proteins for the prediction of ligand affinities.

Notes





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### P-107. Automated and optimally FRET-assisted structural modeling

Mykola Dimura[1,2], Claus Seidel[1], Holger Gohlke[2,3]

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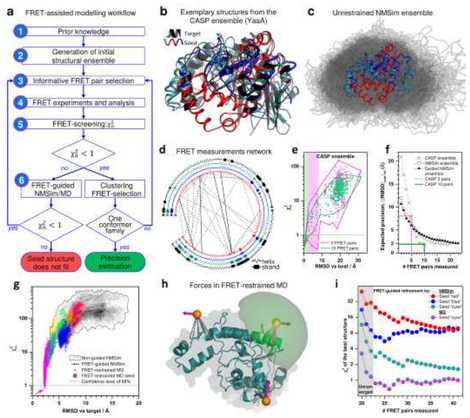
[2]Heinrich-Heine-Universität Düsseldorf, Computational Pharmaceutical Chemistry and Molecular Bioinformatics, Düsseldorf, Germany
[3]John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), and Institute for Complex Systems - Structural Biochemistry (ICS-6), Forschungszentrum Jülich, Jülich, Germany

FRET experiments can yield state-specific structural information on complex dynamic biomolecular assemblies. However, FRET experiments need to be combined with computer simulations to overcome their sparsity. We introduce (i) an automated FRET experiment design tool determining most informative FRET pairs for structural modeling (https://github.com/Fluorescence-Tools), (ii) a protocol for efficient FRET-assisted computational structural modelling at multiple scales (http://nmsim.de), and (iii) a quantitative quality estimate for judging the accuracy of determined structures.

**optional**

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### P-108. Vibrational energy exchange reveals important protein's function signatures

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The exchange of vibrational energy in proteins is crucial for protein function. Changes of Vibrational Energy Exchange are associated with allostery, conformational rearrangement and with heat dissipation subsequent to a chemical reaction. Both computational and experimental studies suggested a connection between Vibrational energy exchange with macromolecule topology. With the present work we study the connection between them considering geometry-based properties such as the proteins" residues coordination number. This relation is proven by molecular simulation in a neuro-pharmacologically relevant transmembrane receptor. Which has been shown to be an allosteric protein. This work helps the study of protein allostery and conformational changes.

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## Poster Presentations

### P-109. How sugars can taste bitter: Insight from multiscale simulations

Mercedes Alfonso-Prieto[1,2], Fabrizio Fierro[1], Alejandro Giorgetti[1,3], Paolo Carloni[1,4]

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[2]Heinrich Heine University Düsseldorf, C. and O. Vogt Institute for Brain Research, Düsseldorf, Germany
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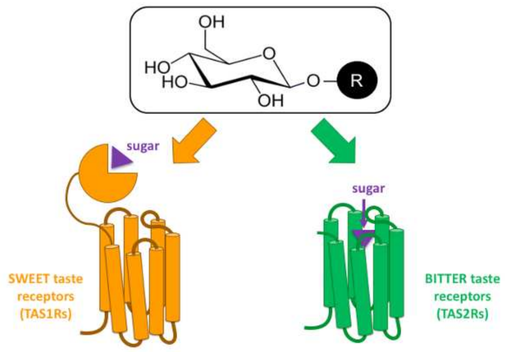
TAS2R16 is a human bitter taste receptor (TAS2R) belonging to the G-protein coupled receptor (GPCR) family, the largest group of membrane proteins in humans. In particular, TAS2R16 is a group-specific bitter taste receptor that is specialized in detecting “bitter sugars”. These bitter glycosides can have either health benefits (such as those present in broccoli and brussels sprouts) or toxic effects (such as the cyanogenic glycosides in bitter almonds and apricot seeds). Therefore, TAS2R16 influences dietary preferences and hence affects human health.

Although originally discovered in the taste buds of the tongue, recently TAS2R16 has been found to be expressed in the human brain and activation by its cognate ligand salicin promotes neuronal growth in neuroblastoma. Furthermore, TAS2R16 genetic variants have been shown to be associated with alcohol dependence, as well as increased longevity. Thus, TAS2R16 is emerging as a promising target for pharmaceutical intervention.

In order to understand TAS2R16 ligand specificity and design novel therapeutic approaches, the first step is to unravel the molecular mechanisms underlying ligand recognition by this receptor. Unfortunately, the lack of three dimensional crystal structures for TAS2R16 (and, in general TAS2Rs) represents a challenge to understand the receptor ligand binding properties at a molecular level. Here we have used bioinformatics and multiscale molecular dynamics to understand the molecular determinants of the ligand specificity of TAS2R16. Our results have been extensively validated by comparison with experiments, including ligand structure-relationship data, mutagenesis and functional data. Given the aforementioned physiological and pathological roles of TAS2R16 mentioned above, this information might also help to design new molecules, either food additives or therapeutic drugs.

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### P-110. Using DOPE inverted hexagonal phase to compare two force decompositions

Otto Schullian[1], Rainhard Lipowksy[1], Markus Miettinen[1]
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The local stress tensor can be calculated from molecular dynamics simulations to gain insight into the mechanical properties of lipid bilayers. A known problem with the current local stress theory is that the solution to the equations determining the stress tensor is not unique. Specifically, different force decompositions give very different results. Some mechanical properties (for example the monolayer bending modulus) can be obtained through other routes, such as applying the Helfrich theory to the inverted hexagonal phase.[1] We calculate the monolayer bending modulus of a DOPE (dioleoyl-phosphoethanolamine) bilayer using the Goetz-Lipowsky[2] (GLD) and Central-Force[3] (CFD) decompositions and compare the outcomes to the values obtained from the inverted hexagonal phase. We find a better match for GLD, but, because of the statistical error, the results are not yet conclusive.

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### P-111. Protein-lipid-ion interactions define conduction properties of TMEM16 lipid scramblases

Andrei Kostritskii[1], Jan-Philipp Machtens[1]
[1]Institute of Complex Systems 4 (ICS-4), Forschungszentrum Jülich, Jülich, Germany

The role of lipids in modulating ion channel functions is becoming increasingly appreciated. Lipids act on the conduction properties in different modes ranging from an indirect influence mediated by the mechanical properties of the membrane to a specific allosteric modulation of ion channel gating. Recent crystallographic and functional data suggest a direct involvement of lipids in ion conduction by lipid scramblases of the TMEM16 protein family. Passively redistributing lipids between the two monolayers of the plasma membrane, these scramblases use a transmembrane hydrophilic cavity, the so-called subunit cavity, as a pathway for polar lipids head groups traversing the membrane. Lining the cavity, these lipid head groups are assumed to form part of a proteolipidic pore permeable for ions, thus being directly implicated in the conduction process.

Here the coupling between ions, lipids, and the subunit cavity was studied by means of all-atom molecular dynamics simulations of ion conduction mediated by the fungal lipid scramblase nhTMEM16. The subunit cavity is shown to have a central localization site for lipid head groups, where they are coordinated by tyrosine Y439. Furthermore, its bulky side chain impedes lipid transitions between extracellular and intracellular parts of the cavity. Confined in this region, lipid head groups demonstrate two distinctive orientational states stabilized by protein-lipid interactions. Importantly, lipids in the cavity, unlike those in the bulk phase, can orient their head groups along the applied electric field. Thus, by influencing head group orientation, voltage polarity affects ion distribution across the channel as well as selectivity of the ionic current. Altogether, our data provide important details of the protein-lipid-ion interplay underlying ion conduction properties in TMEM16 lipid scramblases.

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## Poster Presentations

### P-112. Simulation of cellular adhesion

Filip Savic[1], Andreas Janshoff[1], Burkhard Geil[1]

[1]Georg-August-Universität Göttingen, Institut für Physikalische Chemie, Göttingen, Germany

The adhesion of cells to the extracellular matrix is an important process in biology. To understand the physical processes involved in the onset of cellular adhesion, especially the lateral organization of adhesion molecules into clusters, we perform Monte-Carlo-Simulations based on a harmonic multi-spring model involving lipid membranes and their physical properties. Local deformation of the membrane in the vicinity of adhesion clusters facilitates cluster growth while a repulsive interaction between clusters arises due to an interplay of membrane bending rigidity and non-specific repulsion. Balance of this interactions, their range, and their relative strength compared to the bond strength govern cluster size and stability in our simulations.

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### P-113. Modeling of multiprotein complex formation

Stefanie Förste[1], Reinhard Lipowsky[1], Sophia Rudorf[1]

[1]Max Planck Institute of Colloids and Interfaces, Theory & Bio-Systems, Potsdam, Germany

The formation of a multiprotein complex, arising from the assembly of multiple peptide chains inside the crowded cell environment, is subject of ongoing research. In contrast to the conventional view that protein assembly is a post-translational process, recent experiments show that protein complexes can also assemble co-translationally, i.e., the different chains may assemble before translation has finished. Here, we investigate under which conditions post translational and/or co-translational assembly can occur. We analyze the influence of different parameters – such as the spatial distance of the translation sites – on the assembly dynamics using a combination of Gillespie simulations and analytical modeling. In particular, we study the cross-over from a co-translational to a post-translational assembly regime.

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### P-114. Conformational dynamics and membrane binding of the guanlyate binding protein mGBP2

Jennifer Loschwitz[1], Xue Wang[1], Bogdan Barz[1], Birgit Strodel[1]
[1]Forschungszentrum Jülich, Institute for Complex Systems: Structural Biochemistry (ICS-6), Jülich, Germany

Guanosine triphosphate (GTP) binding proteins (GBPs), which belong to dynamins and dynamin-like protein subfamilies, play an essential role in many cellular processes, such as scission of newly formed vesicles from the membrane of one cellular compartment and fusion with another compartment at the cell surface or the Golgi apparatus. GBPs are interferon-γ induced effector molecules acting against intracellular bacteria and parasites. GTP can bind to murine GBPs (mGBPs) and promote the formation of dimers and also larger multimers, which were demonstrated to destabilize the parasitophorous vacuole (PV) membrane after Toxoplasma gondii infection. In order to obtain an understanding about this process for mGBP2 on the molecular level, biophysical studies of the conformational dynamics of the protein, its dimerization and interaction with lipid bilayers mimicking the PV membrane are required. To this end, we performed Hamiltonian replica exchange molecular dynamics (H-REMD) simulations to elucidate the dynamics of mGBP2 as apo protein, with GTP bound as well as under the influence of both GTP and post-translational geranylgeranylation. In parallel, we studied the binding of mGBP2 to a POPC (1-palmitoyl-2-oleoylphosphatidylcholine) membrane using ordinary molecular dynamics (MD) simulations on the microsecond timescale, which allows us to compare the dynamics of mGBP2 in water and its membrane-bound state, to unravel the effects of mGBP2 on the various membrane properties, and to identify key residues for membrane binding. The current conclusions are that the large-scale motions observed for mGBP2 in water are still present, yet to a limited degree, when bound to the membrane, which in turn causes a disordering and bending of the POPC membrane. In addition to the geranylgeranyl moiety, which inserts into the bilayer upon membrane binding, we identified three lysines as key residues for this process as they significantly interact with the negatively charged heads of the lipid bilayer. Mutation studies testing the relevance of these three lysine residues for the defense against T. gondii infection are under way.

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### P-115. Replica-exchange simulation of T72/S111-Phosphorylated Rab8a GTPase indicates stabilization of the active form

Danial P. Dehkordi[1], Martin Zacharias[1]
[1]Technische Universität München, München, Germany

Rab GTPases constitute the largest branch of Ras protein superfamily that regulate intracellular membrane trafficking. They switch between “active” –GTP-bound– and “inactive” –GDP-bound– forms that are distinguished by closed and open conformation of their two characteristic switch regions. The conformation of the switch regions is central to the recognition of the Rab by its interacting partners, namely, guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) that accelerate the switch cycle. Recent studies have indicated that bacterial pathogens or protein kinases associated with Parkinson”s disease exert post-translational modifications (PTMs) –such as phosphorylation, phosphocolination or adenylylation– on Rabs. The structural and dynamical implications of two of these modifications is addressed in this work using a biasing potential-replica exchange molecular dynamics (BP-REMD) method that penalizes the low energy backbone and side-chain dihedral angles of the protein. We investigate the conformational flexibility of the wild-type, T72- and S111-phosphorylated Rab8a using BP-REMD. Based on our observations, the presence of additional phosphate group intrinsically stabilizes Rab8a in the active conformation. Moreover, we assess the interaction energy between Rab8a and its cognate GEF –Rabin8– and discuss the possible reasons for the interaction impairment. Our model accelerates transitions to Rab8a”s inactive allowing a statistical analysis of the unfolding events in addition to providing means to compare other members of Ras superfamily whose inactive GDP-bound structures have not been resolved yet.

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### P-116. Role of physiological environments in the folding mechanism of intrinsically disordered proteins

Suman Samantray[1], Birgit Strodel[2]

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[2]Institute of Theoretical and Computational Chemistry, Heinrich Heine University Dusseldorf, Düsseldorf, Germany and Forschungszentrum Jülich GmbH, Germany, Computational Biochemistry, ICS-6: Structural Biochemistry, Forschungszentrum Jülich GmbH, Germany, Jülich, Germany

Intrinsically disordered proteins are referred to proteins which natively lack a well-defined structure under physiological conditions. Liquid interfaces such as air-water interfaces (AWI) have been long recognized as environments that enhance protein fibrillation [1]. This is in part due to the interface templating protein conformations favourable for fibril formation. In this work, we use molecular simulations to investigate the behaviour of fragments of amyloid beta, a model fibril-forming protein, at AWI. Exploration of the conformational space is achieved by using enhanced sampling methods, such as replica exchange with solute tempering [2]. We utilize parallel tempering metadynamics simulations [3] to reconstruct the free energy landscape of the amyloid beta 16-22 fragment. Experimental studies have suggested that the 16-22 sequence, which includes the residues 17-21 constituting a hydrophobic core, is essential towards fibril formation [4]. This work encompasses a comparison between proteins in bulk solution and at AWI using an all-atom force field (FF) and a united-atom FF, highlighting the effect of interfacial adsorption on protein conformation, leading to fibrillation. The aforementioned simulation strategies are then applied to human islet amyloid polypeptide (hIAPP) protein, implicated in type-II diabetes.

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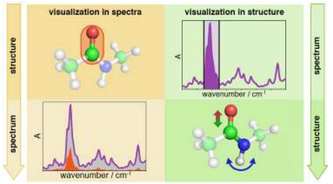
### P-117. Integrating experiment and simulation: decoding IR spectra by visualizing molecular details


Till Rudack[1], Matthias Massarczyk[1], Jürgen Schlitter[1], Carsten Kötting[1], Klaus Gerwert[1]

[1]Ruhr University Bochum, Biophysics, Bochum, Germany


Integration of experimental and computational approaches to investigate chemical reactions in proteins has proven to be very successful. Experimentally, time-resolved FTIR difference-spectroscopy is highly sensitive to minimal structural changes. Structural features are decoded and represented in a comprehensible manner by combining FTIR spectroscopy with biomolecular simulations. We have developed a novel method called local mode analysis (LMA) for calculating IR spectra and assigning spectral IR-bands on the basis of movements of nuclei and partial charges of a QM/MM trajectory. LMA correlates the simulated motions of atoms with the corresponding IR bands and provides direct access to the structural information encoded in IR spectra. Either the contributions of a particular atom or atom group to the complete IR spectrum of the molecule are visualized, or an IR-band is selected to visualize the corresponding structural motions. Applying LMA in combination with FTIR measurements we have identified precatalytic states in the Ras catalysed GTP hyrdrolyses reaction. The obtained detailed structural insights into the catalytic binding pocket of these precatalytic states pave the way to gain a better understanding of oncogenic Ras mutants.

All in all, LMA decodes detailed information contained in complex IR spectra and thereby transforms complex experimental data only accessible by experts into comprehensible easy accessible structural models for a broader science community. Our LMA Graphical User Interface brings theoretical IR spectroscopy in an easy-to-use manner to experimental labs and provides an intuitive approach for structural biologists and biochemists.

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### P-118. Large scale simulations of cell resolved tissue by a cellular Potts model

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Multicellular organisms all face the same fundamental challenge during tissue development: the generation of distinct cell types, structures and organs at specific positions in the body. Advanced imaging techniques provide detailed insight into processes on a single-cell and sub-cell level. Still, a thorough mathematical model of cellular dynamics and differentiation has not been identified. Cellular Potts models are a commonly used tool to describe the cellular behavior. Due to the large computational effort the scale of those simulations has so far been very limited to small and mostly two-dimensional simulation setups. Here, we developed a framework with a fully parallelized implementation of the cellular Potts model, enabling simulations orders of magnitude larger than possible to date and improvements on spatial isotropy. Through parallelization our framework is able to run effectively on large cluster architectures making the simulation of up to $10^9$ cells possible. We present our simulations of tumorigenesis and tumor internal signaling with explicit modeling of the surrounding tissue. Benchmarking with experimental data, such as light sheet microscopy images, becomes possible as the simulated areas reach experimentally relevant sizes. Therefore, our framework is a promising tool to study effects of medication, formation of metastasis and other tissue internal processes to identify underlying base principles.

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### P-119. Precision DEER distance measurements by spin-label ensemble refinement

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Double electron-electron resonance (DEER) experiments, which are also referred to as pulsed electron-electron double resonance (PELDOR) experiments, probe nanometer-scale distances in spin-labeled proteins and nucleic acids. To relate the measurements to distances between molecular sites, the covalently attached spin labels are modeled by rotamer libraries. Here we show that reweighting of rotamer states is essential for precision distance measurements, making it possible to resolve Ångstrom-scale protein domain motions. We analyze extensive DEER measurements on the three N-terminal polypeptide-transport-associated (POTRA) domains of the outer membrane pore Omp85. Using the “Bayesian Inference Of ENsembles’’ (BioEn) maximum-entropy method, we extract rotamer weights directly from the DEER/PELDOR measurements. Already small weight changes eliminate otherwise significant discrepancies between experiment and model, and unmask 1-3 Å domain motions relative to the crystal structure. Rotamer-weight refinement emerges as a simple yet powerful tool for precision distance measurements using pulsed electron paramagnetic resonance experiments. Modeling rotamer states by ensemble refinement is general and should be applicable to other label-based measurements such as paramagnetic relaxation enhancement (PRE) and fluorescence resonance energy transfer (FRET).

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### P-120. Histidine protonation and its influence on the electronic and vibrational properties of a "Rieske-like" iron-sulfur protein

Hendrik Auerbach[1], Kathrin Stegmaier[1], Lena Scherthan[1], Kevin Jenni[1], Jennifer Marx[1], Aleksandr I. Chumakov[2], Antonio J. Pierik[1], Volker Schünemann[1]

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Iron-sulfur (Fe-S) clusters play a central role in energy transduction and electron transport. Unlike "ferredoxin-like" Fe-S clusters, Rieske centers contain a 2Fe2S cluster with one Fe coordinated by two histidines ($Fe_N$) and one Fe coordinated by two cysteines ($Fe_S$). A special feature of the Rieske centers is the pH dependence of their reduction potentials. Protonation of the $N^{\varepsilon 2}$ atoms of the two imidazole rings coordinated to the Fe-S cluster is coupled with cluster reduction (electron transfer) in the Thermus thermophilus Rieske protein. Here, we present a nuclear forward scattering (NFS) and nuclear inelastic scattering (NIS) study on a "Rieske-like" Fe-S protein from Saccharomyces cerevisiae in order to investigate the influence of protonation on the electronic structure of the Fe-S cluster and on the Fe-ligand vibrations. NIS data sets of $^{57}Fe$ enriched "Rieske-like" Fe-S protein at three different pH values (6.4, 8.5 and 10.4) reveal pH dependent vibrational bands in an area where the $Fe_N$-histidine modes occur. In order to explain this effect and to get a deeper understanding of the coupling of the electron transfer to the protonation state of the coordinating histidines, NIS data have been simulated by means of combined quantum chemical and molecular mechanics (QM/MM) calculations based on a model for a 2Fe2S "Rieske-like" cluster with different His-ligand protonation states.

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### P-121. Residues involved in the protonation of the biliverdin chromophore of Agp2

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Agp2 is a bacterial phytochrome from the plant pathogen Agrobacterium tumefaciens. It belongs to the group of bathy phytochromes, which, unlike prototypical phytochromes, have a Pfr dark state with the biliverdin (BV) chromophore in the ZZEssa conformation. It changes due to photo-isomerisation to ZZZssa in the first step of the photoconversion to the Pr state. During the transition from Pfr to Pr, several (de)protonation events take place at the BV chromophore. Tyrosine 165 and arginine 211 are strongly conserved among all phytochromes and are located in the chromophore-binding pocket in close proximity to the essential protonation sites of the biliverdin chromophore. FT-Raman and FTIR measurements on Agp2-Y165F and -R211A variants showed that the photocycle was incomplete in both variants, going as far as meta-F intermediate state. The deprotonation of the propionic side chain of the BV-ring C was impaired and no keto-enol tautomerization was observed at the carbonyl group of ring D. The latter is pH-dependent in the wild type Agp2 and was proposed to be essential for the dark Pr-to-Pfr reversion. UV-vis spectroscopic kinetic measurements showed that indeed dark reversion was extremely slow and pH-independent in the Agp2-Y165F variant, while the R211A variant showed a behavior much more similar to the wild type protein. Based on the presented results as well as structural data we propose specific roles for the two amino acid residues. Y165 helps to conduct the immediate deprotonation of the ring C propionic side chain, while R211 locks the deprotonated propionate residue thus preventing it from re-protonation in a reverse process.

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### P-122. Immobilization of cytochrome c oxidase for spectro-electrochemical investigation

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Cytochrome c Oxidase (CcO) plays a vital role in most aerobe living organisms. As the terminal enzyme of the respiratory chain it catalyses the last step of electron transport by reducing molecular oxygen to water. The energy gained from this process is harnessed by pumping additional protons across a membrane, building up a gradient, which can in turn be used by the ATP-synthase for ATP recovery. Several methods have been developed to study the well-orchestrated mechanism of electron and proton delivery for the pumping process as well as the catalytic reaction, which is devoid of unwanted side reactions, e.g. hydrogen peroxide formation.

To specifically probe the active site of the enzyme, without an overflow of information from the entire enzyme, Resonance Raman spectroscopy is an excellent tool. To further increase signal intensities, the surface enhancement effect of some electrode surfaces can be used. For this, immobilization of CcO is crucial but additionally enables direct electron transfer between electrode and enzyme for simultaneous electrochemical studies with the same setup.

Using an amino terminated Self-assembled monolayer (SAM), imitating the lysine rich binding pocket of Cytochrome c, good spectroscopic signals were achieved, but voltammetric measurements were not possible with this system. A novel approach to this problem is the use of a hydrophobic SAM, which is formed under applied potential, thus yielding a better coverage of the electrode surface compared to the patchy patterns of the amino terminated SAMs.

Cyclic voltammetry of the dodecanethiol-SAM revealed a good immobilization of CcO, showing a distinctive reduction signal and oxidation peaks, which are separable by lowering the scanrate. Spectroscopic measurements of the immobilized enzyme were performed to study the structure of the active site and changes thereof under the influence of different applied potentials.

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### P-123. Time-resolved single-frequency infrared-spectroscopy on photosystem II in $H_2O$ and $D_2O$: tracking protonation dynamics

Sarah Mäusle[1], Philipp Simon[1], Holger Dau[1]
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Photosystem II (PSII) is a large protein complex in plants and cyanobacteria which catalyzes the oxidative splitting of two water molecules into four electrons (reducing equivalents), four protons, and one dioxygen molecule. Following the absorption of a photon, charge separation takes place at a special chlorophyll pair, P680, allowing an electron to move to the acceptor side of PSII. The resulting positive hole leads to the oxidation of a redox-active tyrosine residue $Y_Z$ and subsequently of the manganese-calcium ($Mn_4Ca$) complex. After accumulating four oxidative equivalents at the $Mn_4Ca$ cluster, dioxygen is formed. The four semi-stable redox states of the $Mn_4Ca$ cluster are referred to as the S-states ($S_0$ to $S_3$) (Dau, H. & Haumann, M. (2008) Coord. Chem. Rev. 252(3-4): 273-295). The process leading up to $O_2$ formation is still not fully understood on an atomic level.

Structural and electrostatic changes that occur in PSII during the S-state cycle influence its vibrational modes and can thus be observed via difference infrared spectroscopy. Our time-resolved single-frequency IR setup allows for the observation of electron transfer as well as protonation dynamics with a high temporal resolution (up to 50 ns). $H_2O/D_2O$ exchange can help to identify events involving proton movement by investigation of the kinetic isotope effect (KIE = $\tau_D/\tau_H$). A KIE exceeding unity suggests involvement of protons, while the specific value can hint at the type of protonation dynamics involved (Krishtalik, L. I. (2000) BBA-Bioenergetics: 1458(1): 6-27).

Time traces at various wavenumbers, such as 1395 $cm^{-1}$ (symmetric $COO^-$ vibrations), 1515 $cm^{-1}$ (CO stretching of $YZ^\bullet$), and 1544 $cm^{-1}$ (Amide II band), were recorded for PSII membrane particles from spinach. The respective time traces were fitted with multi-exponential functions and the extracted time constants were then used to calculate the KIE, allowing us to identify and analyze protonation dynamics that eventually lead to $O_2$ formation.

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### P-124. The influence of water analogues on the oxygen-evolution step in Photosystem II

Ricardo Assunção[1], Holger Dau[1]

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Photosystem II (PSII) harbors an active site Mn4Ca-oxo cluster that facilitates the multi-step water oxidation reaction involving four events of excitation by light, intercalated by electron transfer and deprotonation steps (Kok"s S-state cycle) [Dau et. al 2012, Curr Opin Chem Biol, 16(1-2), 3-10]. Being water both the solvent and the substrate for this reaction, the detailed mechanistic aspects become very demanding to investigate and are currently still insufficiently understood. In particular, the likely crucial role of the water cluster neighboring the metal-oxo core at the catalytic site and the identity of the 'substrate' water molecules, which is closely related to the identification of the actual O-O bond formation mechanism, are still unknown [VINYARD et. al 2017, Annu Rev Phys Chem, 68]. The use of water analogues, molecules sufficiently similar to water that could either replace substrate water molecules or other water molecules in the vicinity of the Mn-complex, like ammonia (NH3) or Methanol (MeOH), are one of the ways to approach this question. NH3 is a reported inhibitor [SCHUTH et. al 2017, Biochemistry, 56(47)] that has at least two binding sites, being at least one directly to the Mn-complex, most likely W1 [NAVARRO et. al 2017, Proc Natl Acad Sci U S A, 110(39)]. Methanol is reported to have two binding sites closely to the Mn-complex, but may not replace any of the substrate waters [NAGASHIMA et. al 2017,J Phys Chem Lett, 8(3)]. Using time-resolved measurements on PSII membrane particles, we aim to understand how water analogues affect the kinetics of the oxygen evolution step.

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### P-125. Single-frequency IR spectroscopy with microsecond time resolution for tracking electron and proton transfer in the D1-V185N variant of photosystem II

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In the light-driven reaction cycle of Photosystem II (PSII), two ‘substrate’ water molecules are oxidized resulting in the release of $O_2$ at the oxygen-evolving complex (OEC), which consists of a $Mn_4Ca$-oxo cluster and its water-protein environment. Driven by a sequence of light flashes, the OEC cycles through its four semi-stable S-state intermediates. These transiently formed states are involved in ill-understood alternating electron and proton transfer steps [Klauss et al. 2012, Proc. Natl. Acad. Sci. U.S.A., 109(40)]. For complete mechanistic understanding of photosynthetic water oxidation, the transiently formed intermediates need to be investigated in detail.

Genetic modification of amino acid residues in the vicinity of the OEC has been repeatedly approached for the cyanobacterium Synechocystis sp. PCC 6803. Such manipulations aim to alter the behavior of the PSII photocycle to help identify the involved mechanisms. Recently, retarded $O_2$ release kinetics, with altered pH and isotope exchange characteristics, have been reported using time-resolved oxygen polarography with an asparagine substitution on the D1-Val185 residue [Bao et al. 2015, Proc. Natl. Acad. Sci. U.S.A., 112(45)].

An important tool to study such protonation and oxidation states changes is infrared spectroscopy. Our novel quantum cascade laser based single-frequency infrared absorption setup produces a time-resolution of 1 µs and a spectral resolution of 0.5 $cm^{-1}$. With a tuning range of 1300 $cm^{-1}$ to 1650 $cm^{-1}$, encompassing much of the characteristic carboxylate and amide II bands, and an integrated ns pulsed 532 nm excitation laser, the time evolution of transitioning PSII complexes can be observed. This contribution describes the early work of a collaboration geared at a comparison of transients of the wild type Synechocystis sp. PCC 6803 and the mutant D1-Val185Asp absorption bands to investigate the dynamics of the water cavity and the OEC.

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### P-126. Time-resolved IR absorption spectroscopy: tracking photosynthetic water oxidation in photosystem II

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Photosystem II (PSII) is one of the two light-activated photosynthetic proteins and performs water oxidation in order to reduce the mobile membrane bound electron carrier plastoquinone. At the catalytic center, a $Mn_4Ca$-oxo cluster, four oxidizing equivalents needed for O-O bond formation are accumulated involving a sequence of alternating steps of electron and proton removal from the catalytic site (Klauss et al., J. Phys. Chem. B (2015), 119, 2677-2689).

Understanding of the process at an atomic level may not only answer basic questions of the light reaction of photosynthesis and PSII function but could also provide hints on the development of improved catalysts for artificial water splitting, a clean way of producing storable energy carriers.

We designed an infrared absorption experiment with a continuous-wave quantum cascade laser tunable from 1300 to 1650 $cm^{-1}$ and thus covering the amide I and II regions, the symmetric and asymmetric $COO^-$ stretching region as well as bands of the quinones and the redox-active tyrosine (denoted as $Y_Z$). The current time resolution is in the tens of ns range, which enables us to observe pivotal structural changes and proton transfer dynamics in PSII.

We will provide an overview of the complex dynamics of PSII trackable at the various wavenumbers. This includes the electron transfer from the tightly bound reduced quinone $Q_A^-$ to the exchangeable quinone $Q_B$, the multiphasic kinetics of the oxidation of $Y_Z$ by the special chlorophyll unit P680 and the proton coupled electron transfer events around the Mn cluster. The latter data is measured on PSII-enriched membrane particles of spinach as well as core complexes of the cyanobacterium Synechocystis sp. PPC 6803. Because we can track all these processes in functional proteins with only one data set, time resolved IR measurements on PSII will provide new insights on their nature in the near future.

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### P-127. Analysis of photoinduced processes of phycoerythrobilin-loaded cyanophage phycobiliprotein lyase ΦCpeT using femtosecond transient absorption spectroscopy

Christopher Carlein[1], Maximilian Theiß[1], Natascha Riedel[1], Florian Mahler[1], Sandro Keller[1], Nicole Frankenberg-Dinkel[1], Rolf Diller[1]

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Phycobiliprotein lyases mediate the chromophore assembly of light-harvesting phycobiliproteins in cyanobacteria (1). Interestingly, some cyanophages - viruses that infect cyanobacteria - also possess genes encoding phycobiliprotein lyases. It has been suggested that they might contribute to increasing photosynthetic efficiency in cyanobacteria during infection (2). The cyanophage P-HM1 encoded phycobiliprotein lyase ΦCpeT forms a stable non-covalent complex with the linear tetrapyrrole phycoerythrobilin (PEB) (2). To better understand how phycobiliprotein lyases might facilitate the phycobiliprotein assembly we studied the interaction of ΦCpeT with its ligand PEB by employing static UV/Vis and fluorescence spectroscopy as well as femtosecond transient absorption and time-resolved fluorescence spectroscopy. This provides insights into the photophysical and photochemical processes after photoexcitation of protein-bound linear tetrapyrroles (3,4) in contrast with their free forms (5,6). Additionally, we investigated ΦCpeT variants with a modified binding pocket (e.g., R55A and D117A) and their influence on the binding properties of PEB.

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### P-128. Conservation and variation of electron transfer in the photolyase–cryptochrome protein family

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We investigate the thermodynamics and kinetics of the sequence of electron transfer (ET) steps taking place in the photo-activation of photolyases (PL) and cryptochromes (CRY). The long-range hole transfer from the photo-excited FAD cofactor to a net electron donor proceeds via a conserved tryptophan triad, and exhibits similar but somewhat different ET patterns. These ET processes and the accompanying structural relaxations of the protein take place on temporal scales that often overlap, which is difficult to tackle by using classical theories of ET. Instead, a flexible and transferable computational scheme free of any assumptions about the ET mechanism is needed to obtain an unbiased description of these processes. To this end, we have developed an efficient multi-scale simulation framework that combines non-adiabatic propagation and linear-scaling quantum chemistry with a molecular mechanics force field. In addition, the main and the alternative ET pathways may be identified by including additional aromatic amino acid residues in the quantum description.

Specifically, the sequential ET in E. coli PL is shown to take place on the same temporal scale as the relaxation of protein, and it is the surrounding solvent that provides a driving force for the transfer, rather than the protein itself. The same observation is made for the formation of a persistent radical pair in A. thaliana CRY 1. A number of ET pathways are explored in PhrB from A. tumefaciens, which is an ancient bacterial 6–4 PL that features a tyrosine in the ET triad, and a combined experimental and computational mutagenesis study characterizes the main ET channel. Further, the intricate thermodynamic and kinetic control of branching pathways is investigated in PhrA, a class-III cyclobutane pyrimidine dimer PL from A. tumefaciens. Finally, the effect of proton transfer coupled to ET on the kinetics of ET itself is described in one representative PL and CRY each.

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### P-129. A theoretical framework for spatiotemporal chemical imaging with nanosensors


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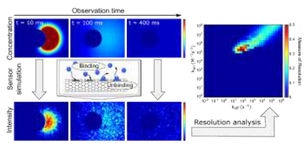
Fluorescent nanosensors are powerful tools for basic research and bioanalytical applications. Here, individual nanosensors are able to detect single molecules, while ensembles of nanosensors can be used to measure bulk concentrations of an analyte or even provide spatial and temporal information of fast cellular release events when imaged collectively. So far, it is not understood which processes are resolvable with this method (concentrations, spatial and temporal resolution) and how an optimal sensor should be designed to attain optimal measurement conditions.

For that reason, we developed a theoretical framework that mimics experimental conditions and simulate the fluorescent image of arrays of nanosensors in response to any spatiotemporal concentration gradient $c_A(x,y,t)$[1]. Using Gillesby based Monte Carlo methods, binding and unbinding of analytes such as the neurotransmitter dopamine were simulated on the single nanosensor level for varying binding rates ($k_{on}$ and $k_{off}$) and used to predict the response images I(x,y,t) of sensor arrays. We introduce terms for the spatial and temporal resolution for such systems and simulate phase diagrams for different rate constants that allow us to predict how a sensor should be designed to provide a desired spatial and temporal resolution. Our results show, for example, that imaging of neurotransmitter release requires rate constants of $k_{on} = 10^6\ M^{-1}s^{-1}$ and $k_{off} = 10^2\ s^{-1}$ in many scenarios, which corresponds to high dissociation constants of $K_d > 100\ \mu M$.


[1]Meyer, D.; A. Hagemann, A.; Kruss, S., Kinetic requirements for spatiotemporal chemical imaging with fluorescent nanosensors. ACS Nano 2017, 11, 4017-4027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### P-130. Spectroscopic characterization of graphene quantum dots for in vivo applications

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Graphene quantum dots (GQDs) are a promising candidate for various biomedical applications ranging from drug delivery to single particle tracking, due to their small size and low toxicity. To characterize GQDs concerning their viability as a fluorescence marker time correlated single photon counting and fluorescence correlation spectroscopy are employed. Measurements indicate that the particles are encapsulated in inclusion bodies when taken up by cells. To release the particles into the cytosol, GQDs are given a positive zeta-potential. The functionalized GQDs and their non-functionalized counterpart are studied after the uptake by human breast cancer cells for different incubation times.

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### P-131. Quantitative analysis of structural and affinity properties of molecular assemblies in living cells using MFIS-FRET studies with fluorescent proteins

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A new general strategy based on multi-parameter fluorescence detection (MFD) is introduced to register and quantitatively analyze fluorescence images. Multi-parameter Fluorescence Image Spectroscopy (MFIS) uses pulsed excitation, time-correlated single-photon counting and a special pixel clock to simultaneously monitor the changes in fluorescence information (fundamental anisotropy, fluorescence lifetime, fluorescence intensity, time, excitation spectrum, fluorescence spectrum, fluorescence quantum yield and distance between fluorophores) in real time. In addition the three spatial coordinates are stored. Statistically most efficient techniques known from single-molecule spectroscopy are used to estimate fluorescence parameters of interest for all pixels and not only for regions of interest. Their statistical significance is judged in a stack of two-dimensional histograms. In this way specific pixels can be selected for subsequent pixel based sub-ensemble analysis to improve statistical accuracy of the estimated parameters. MFIS avoids sequential measurements, because the registered data allows one to perform many analysis techniques such as fluorescence-intensity distribution analysis (FIDA) and fluorescence correlation spectroscopy (FCS) in an off-line mode.

To demonstrate the ability of our technique, we used intramolecular FRET experiments between fluorescent proteins (FPs, eGFP and mCherry) in combination with Monte Carlo simulations to parameterize fast coarse-grained accessible volume simulations of FPs in plant cells and mouse fibroblasts, respectively. This allows us to relate structural features and affinities of molecular complexes with intermolecular MFIS-FRET measurements.

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### P-132. Studying biomolecular systems beyond the diffraction limit with molecular resolution by STED-MFIS microscopy

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We combine Stimulated Emission Depletion (STED) microscopy and Multiparameter Fluorescence Image Spectroscopy (MFIS) to selectively measure and characterize biomolecular systems on surfaces and in living cells with molecular resolution. Scanning confocal STED microscopy overcomes the diffraction limit and localizes molecules with a resolution down to 25 nm, while MFIS monitors simultaneously a variety of fluorescence parameter such as fluorescence intensities, fluorescence lifetimes and anisotropy pixelwise in three spatial dimensions (3D). MFIS allows for detailed spectroscopic analysis and provides Ångström resolution via Förster Resonance Energy Transfer (FRET). Thus, macromolecules can be localized in living cells with nanometer accuracy (STED), while monitoring their structure with Ångström resolution (FRET). Hetero-FRET studies on model systems with small synthetic fluorophore and fluorescent proteins, respectively, demonstrate the benefits of combining STED and MFIS techniques to map the localization of macromolecules with high precision and to resolve their inter- and intramolecular structural and dynamic features simultaneously. In a test case, we systematically vary distance within a FRET pair up to the practical resolution limits of STED. This demonstrates that a combination of STED and FRET bridges the length scales of FRET-spectroscopy and microscopy to study cellular architectures in living cells with Ångström resolution.

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### P-133. Quantitative ultra-fast FLIM

Maria Loidolt-Krüger[1], Fabian Jolmes[1], Marcelle König[1], Rhys Dowler[1], Paja Reisch[1], Ben Krämer[1], Marcus Sacrow[1], Matthias Patting[1], Tino Tino Roehlicke[1], Hans-Juergen Rahn[1], Michael Wahl[1], Felix Koberling[1], Rainer Erdmann[1]
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Increasing the speed of Fluorescence Lifetime Imaging (FLIM) is essential to cementing its importance as a tool in the Life Sciences. This technique is already well established, but imaging dynamic processes requires shorter acquisition times. Our novel rapidFLIM approach dramatically reduces the acquisition time through a combination of fast scanning, hybrid photomultiplier detectors which are capable of handling very high count rates, and TCSPC modules with ultra short dead times. With the new FLIMbee fast scanning add-on for the MicroTime 200, this technique can be used with our microscopy platform as well as being offered as an upgrade kit for conventional Laser Scanning Microscopes (LSMs).
With this hardware combination, excellent photon statistics can be achieved in significantly shorter time spans, allowing fast processes to be measured with the high resolution achieveable in confocal microscopy. Depending on the image size, rapidFLIM allows imaging at a rate of several frames per second, enabling dynamic processes, such as protein interactions, FRET dynamics, or chemical reactions to be imaged in a time-resolved manner. With these high frame rates, FLIM can also be used on highly mobile species such as cell organelles and for other live cell imaging applications.
Recently, we have further pushed the limits of this method by systematically reducing the effects of decay distortions at very high count rates, allowing quantitative data analysis to be performed even at count rates >> 10 Mcps. This technique has been applied to quantitatively analyze FRET measurements using fluorescent proteins.

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### P-134. Fast and efficient fluorescence data acquisition for high throughput, kinetics and imaging applications

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Fluorescence has a multi-parameter signature in terms of wavelength, intensity, polarisation, lifetime and position. Ideally its measurement should be rapid and efficient. The environmental sensitivity of fluorescence makes it an ideal probe for monitoring changes / interactions on the nanoscale and recent technological advances has enabled parallel acquisition of intensity – wavelength data. For example, the collection of excitation-emission matrix (EEM) surfaces can be made in a fraction of the time with simultaneous acquisition of all emission wavelengths. Combining fast fluorescence acquisition and the sample absorption allows to correct for the inner filter effect and is termed A-TEEM(TM) (absorbance-transmission and fluorescence excitation emission matrix ) method. Applications include monitoring complex sample composition and protein therapeutics.

Time-resolved measurements have also benefited from these advances. Very low deadtime (<10ns) electronics coupled with high frequency (100MHz) laser excitation enables efficient fluorescence lifetime determination using time-correlated single-photon counting (TCSPC). Photon streamed (aka “time tag”) data is useful for monitoring kinetic processes, such as small molecule – protein binding; further enhanced by the use of high quantum efficiency hybrid detectors. Single-photon avalanche photodiode (SPAD) arrays with associated TCSPC electronics fabricated using CMOS technology have enabled the use of widefield fluorescence lifetime imaging (FLIM) cameras with nearly 25,000 pixels to be employed. This provides data collection orders of magnitude faster than traditional scanning fluorescence microscopy and this “fast-FLIM” opens the way for near video rate fluorescence lifetime imaging.

In this work we will demonstrate with application examples the use of steady state and time-resolved measurements, including “fast-FLIM”, to rapidly and efficiently collect a more complete measurement of the fluorescence signature.

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### P-135. Uptake and release of proteins in microgels studied on single particle level

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For efficient drug delivery, a smart network of polymers called microgel particles can be used. These particles with an ability to swell and de-swell in response to environmental changes, allow uptake and release of drugs in a controlled manner. Their sensitivity to environmental changes along with biocompatibility make them a good candidate for drug carrier, especially for therapeutic proteins. An impact of environmental conditions on proteins outside the cell often causes protein denaturation and aggregation. We studied the uptake and release of the positively charged cytochrome c, in and from microgel particles.
The loading of the microgels took place at pH 8, a value at which microgels are charged negatively. By employing wide-field fluorescence microscopy we made a reliable characterization of single particles, e.g. the maximum loading of the particles, the stability of the loading, and the amount of proteins to be released at the target location.

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### P-136. Direct characterization of the evanescent field in total internal reflection fluorescence microscopy

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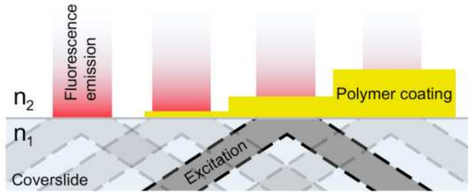
Total internal reflection fluorescence microscopy (TIRFM) offers highest surface specificity, based on the assumed single-exponential evanescent field. However, quantitative interpretation of the axial information in TIRFM images requires the accurate knowledge of the excitation profile. We devised simple and low-cost calibration samples by coating coverslides with a staircase-like profile of a polymer with a refractive index matching that of water. The slides have a long shelf time and can be used on standard TIRFM setups without special equipment. Using our calibration slide, we not only found an exponential decay in good agreement with theoretical predictions, but also characterized a non-evanescent contribution often described for objective-type TIRFM, which is supposedly caused by scattering within the optical path or optical aberrations. Here, we present the application of our calibration slide for the routine calibration of TIRFM setups and describe its implementation for quantitative TIRF imaging along the axial dimension. Furthermore, we explore the adaption of the calibration slides for 3D single-molecule localization microscopy, where the axial localization typically requires an initial point spread function calibration by imaging emitters in different heights above the coverslide.

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### P-137. Fast near infrared imaging of dopamine with fluorescent nanosensors

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Quantifying concentration changes of molecules with high temporal, spatial and chemical resolution is one of the great challenges in microscopy. Our approach to achieve this goal is to create nanoscale fluorescent sensors that report about their local chemical environment. Single nanosensors can be used to study chemical processes on the single-molecule level while imaging of many sensors provides spatial information.

In our work we use nanomaterials to synthesize such sensors and focus on near infrared (nIR ) fluorescent materials because this spectral region provides many advantages. Here, we use semiconducting single-walled carbon nanotubes (SWCNTs) as non-bleaching nIR fluorescent building blocks. To create a sensor both photophysics and molecular recognition need to be understood and controlled. Therefore, we have developed different functionalization schemes to design the organic phase around these nanomaterials. These approaches include non-covalent peptide, DNA or polymer adsorption but also incorporation of sp3 defects and covalent conjugation strategies.

Photophysical changes of these sensors were analyzed by using nIR fluorescence spectroscopy and microscopy. Most notably, we gained a deeper understanding of how fast and how strong single molecules such as dopamine interact with the organic phase and the nanomaterial. These experimental kinetic insights were supplemented by kinetic Monte-Carlo-Simulations that provide a full theoretical framework for chemical imaging with nanosensors.

As an application arrays of these sensors were used for spatiotemporal chemical imaging release of small biomolecules from cells. One example includes imaging fast neurotransmitter (dopamine) release on the milliseconds time scale and identification of exocytosis hot spots.

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### P-138. Localization-based fluorescence correlation spectroscopy with DNA-PAINT

Johannes Stein[1], Florian Stehr[1], Philipp Blumhardt[1], Julian Bauer[1], Patrick Schüler[1], Florian Schueder[1,2], Jonas Muecksch[1], Ralf Jungmann[1,2], Petra Schwille[1]

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The field of super-resolution (SR) microscopy has provided life scientists with a diverse array of light microscopy techniques for investigating biological structures far below the classical diffraction limit. DNA-PAINT (points accumulation for imaging in nanoscale topology) employs fluorescently labeled single-stranded DNA oligonucleotides, so called imagers, which reversibly bind to complementary docking strands tethered to a biological target of interest. The resulting “blinking” allows stochastic and localization-based rendering of super-resolved images. One advantage of DNA-PAINT is that the blinking-related imager binding kinetics can be recovered from these super-resolved images which in turn allows for further quantitative analyses. In this work based on DNA-origami we apply the principle of fluorescence correlation spectroscopy (FCS) to DNA-PAINT data. We present a robust analysis tool for obtaining imager dynamics and demonstrate that our method allows molecular counting even in dense clusters of docking sites.

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### P-139. Single-Particle Tracking based on DNA-Paint

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Single-particle tracking by fluorescence microscopy is a powerful technique to study the motion and organization of proteins and lipids in the plasma membrane that has reshaped our image of biological membranes over the past 20 years. Organic fluorescent dyes are widely used for labelling the proteins or lipids in these experiments because these dyes are small, more photo- stabile than fluorescent proteins, and they can be side-specifically coupled to the protein or lipid of interest. However, the finite photon budget of these dyes is still a limiting factor, since the motion of a single molecule (dye) can only be followed for a few hundred time points (typically a few seconds) before photo-bleaching occurs. Here, we present a method based on DNA-PAINT (points accumulation for imaging in nanoscale topology) that can overcome this limitation and therefore potentially allows to track single particles for arbitrary long periods at high resolution. In DNA-PAINT, a stochastic super-resolution microscopy technique based on single-molecule localization, biological targets are labeled with single-stranded oligonucleotides (docking strands) to which complementary fluorescently-labeled oligos (imagers) reversibly bind. For super- resolution post-processing, these binding events have to be sparse in order to record the positions of a non-overlapping subset of bound imagers in each raw image.
For the purpose of single-particle tracking we now reverse this principle by designing a docking strand that is permanently visited by at least one imager at the time ensuring that imagers are continuously replaced. Using click-chemistry for tethering our docking strands to molecules of interest has the potential to combine efficient 1-to-1 labeling with long-term tracking of molecules, both in in vitro model membrane systems and in the plasma membrane of live cells.

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### P-140. Zinc inhibition in the NpHV1 voltage-gated proton channel

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Zinc is a physiological inhibitor of voltage-gated proton channels (HV1). A substantial number of reports have shown that inhibition of HV1 by zinc changes the physiology of several cell types; e.g. diminishing the oxidative burst in phagocytes, limiting histamine secretion in basophils, reducing invasiveness of cancer cells, and preventing the final maturation of human sperm. We investigated the zinc inhibition of a new member of the voltage-gated proton channel family, NpHV1, from the insect Nicoletia phytophila. We found that NpHV1 is inhibited less by external zinc than the human channel hHV1 at pHo 7. Both slowing of activation (τact) and the shift in voltage threshold are diminished. Aligning the insect channel with the human channel reveals that NpHV1 possesses only one of the two externally accessible histidines. Instead of a histidine in the second extracellular loop NpHV1 expresses an aspartate (Asp145). Asp has a very low pKa value which leaves it deprotonated in solution at pHo 5. The effects of zinc were abolished at pHo 5. This contradicts the hypothesis that Asp permits channel inhibition even at low pH. Substitution of Asp145 with His145 restores zinc sensitivity of NpHV1 comparable to hHV1. Substitutions of the His92 to Ala92 and His92/Asp145 to Ala92/Ala145 render the channel almost zinc insensitive. The data support the idea that strong zinc inhibition of the proton channel is dependent on a histidine in the S3-S4 linker.

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### P-141. Detailed analysis of zinc inhibition in the voltage-gated proton channel

Gustavo Chaves[1], Arne Franzen[2], Stefanie Bungert-Plümke[2], Boris Musset[1]

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The $H_V1$ voltage-gated proton channel is a key component of the cellular proton extrusion machinery and is pivotal for charge compensation during the respiratory burst of phagocytes. The best described physiological inhibitor of voltage-gated proton channels ($H_V1$) is $Zn^{2+}$. Externally applied $ZnCl_2$ drastically reduces recorded proton currents in Homo sapiens, Rattus norvegicus, Mus musculus, Oryctolagus cuniculus, Rana esculenta, Helix aspersa, Ciona intestinalis, Coccolithus pelagius, Emiliania huxleyi, Danio rerio and Lingulodinium polyedrum. There is an ongoing discussion where the potential $Zn^{2+}$ coordination sites are located. Recent studies in the proton channel from Nicoletia phytophila, $NpH_V1$, confirm a slowing of the activation-time constant and a positive shift of the conductance-voltage curve. $Zn^{2+}$ inhibition in different extracellular pH, with $Zn^{2+}$concentrations ranging from 0 µM to 1000 µM, and mutations done at potential zinc coordination sites demonstrate a smaller inhibitory effect  than reported previously in mammalian $H_V1$. Replacing an aspartate in the S3-S4 loop with a histidine caused both the slowing of activation and the shift in the voltage-conductance curve to approach the $Zn^{2+}$ inhibition reported for the human channel. The latest data suggest that histidine and not aspartate in the S1-S2 and S3-S4 linker coordinates $Zn^{2+}$ to inhibit voltage-gated proton channels.

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## Poster Presentations

### P-142. Supramolecular Assembly of Lipid Nanodiscs using Genetically Engineered MSP1D1 for Membrane Protein Structural Studies

Madhumalar Subramanian[1,2], Karim Fahmy[1], Jana Oertel[1]

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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 carrying 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 disulfide linkage, thereby creating a more ordered system for membrane protein structural studies.

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 of the nanodisc. Different strategies were investigated to create nanodisc dimers for XFEL-based structural studies.

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### P-143. Structural changes of the oxygen-evolving complex towards the $O_2$ formation

Mohamed Ibrahim[1], Jan Kern[2], Ruchira Chaterjee[2], Iris Young[2], Franklin Fuller[2], Athina Zouni[1], Holger Dobbek[1]
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Humankind was always inspired by nature to develop new tools, which contributed to our civilization on Earth. Cyanobacteria is the first organism to create the machinery to efficiently use the solar energy for building up and storing food. The dioxygen that we are breathing is a byproduct of this mechanism, which now known as photosynthesis. Understanding the fundamentals of photosynthesis could provide a platform for the scientists to develop the tools that could sustain our civilization on Earth. The main player in the photosynthesis is Photosystem II (PSII), the multi-subunit membrane protein that contains the oxygen-evolving complex (OEC), where the Mn4CaO5 cluster cycles through five different intermediate S-states (S0 to S4), and the dioxygen is released after each complete cycle. Hitherto, the mechanism of water photo-oxidation is still elusive, and more structural information for each S-state is required to understand it.

Serial femtosecond X-ray crystallography (SFX) using an X-ray free electron laser (XFEL) was used to investigate the structural changes of PSII during the water oxidation process. XFEL provides very short X-ray pulses (< 50 fs) giving a chance for recording the diffraction at room temperature (RT) before the onset of radiation damage. For this, I developed a new protocol for producing microcrystals of the PSII that can be used at the SFX. The damage free RT high-resolution structures of PS II (2.04-2.08 Å) in all Kok"s cycle meta-stable states and of two time-points, at 150 and 400 µs, during the transition S2→S3 state (2.20-2.50 Å) are reported. Suggesting a direct involvement of one water ligand (W3) and one oxo bridge (O5) of the OEC in O-O bond formation and/or substrate delivery.

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### P-144. Detailed Comparison of $H_2O_2$ Production of Human PMN and HL-60 derived Cell Lines.

Annika Droste[1], Gustavo Chaves-Barboza[1], Stefan Stein[2], Matthias Schweizer[3], Boris Musset[1]

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[3]Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Langen, Germany

The interplay between the voltage-gated proton channel and the activity of the NADPH oxidase is still unsolved. Several attempts have been made utilizing numerous inhibitors, blockers, ionophores, and activators to elucidate the interconnection. We plan to decipher this question by genetic manipulation of voltage-gated proton channels in PMN. The oxidative burst of human PMN and human cell lines are compared to identify the most suitable cell line representing PMN for further genetic manipulation. Here, we are using a fluorescence-based assay to measure $H_2O_2$ production from human PMN and four cell lines representing human PMN. Activation kinetic and maximal production are compared as well as inhibition due to zinc. NADPH oxidase is not affected by zinc concentrations used in the assay. However, voltage-gated proton channels are inhibited by [$Zn^{2+}$] applied. Our results strongly suggest that voltage gated-proton channels modulate ROS production. Furthermore, effects of zinc on human PMN and cell lines are identical, supporting a proposed general mechanism of oxidative burst in human phagocytes.

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### P-145. Spectroscopic investigation of variants of channelrhodopsin-1 from chlamydomonas augustae

Maria Walter[1], Raiza Maia[1], Vera Muders[1], Joachim Heberle[1], Ramona Schlesinger[1]

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Channelrhodopsins are photoreceptors located in the eye-spot of green algae, which cause phototactic responses depending on light conditions in the surrounding. Channelrhodopsin-2 from Chlamydomonas reinhardtii (CrChR2), a light-gated cation channel, is used in the neurophysiological field to optically control cellular processes. Channelrhodopsin-1 from Chlamydomonas augustae (CaChR1) is yet another promising optogenetic tool with mechanistic differences to CrChR2. To understand which amino acids are involved in the process of channel opening upon light activation, we created variants of CaChR1 and analyzed them by means of time-resolved UV-vis spectroscopy and FTIR difference spectroscopy. Here, especially we focused on the role of cysteines.

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### P-146. Spectroscopic investigations on the light-driven inward $H^+$ pump xenorhodopsin

Luiz Schubert[1], Ramona Schlesinger[1], Joachim Heberle[1]
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Ion gradients across the cell membrane drive the synthesis of adenosine triphosphate (ATP). In several archaea, bacteria and unicellular eukaryotes these ion gradients are established by light-driven rhodopsins. Recently, a new class of proton-pumping bacterial rhodopsins, named xenorhodopsin (XeR), has been discovered. Although it has been shown, that the crystal structure of XeR from Nanosalina (NsXeR) is similar to the outward proton pump bacteriorhodopsin (bR), the vectoriality of proton translocation is inverted.

We investigated NsXeR by means of time-resolved spectroscopy to get an insight in it´s photocycle. UV/Vis flash photolysis experiments indicate at least 4 intermediates. Similar to the photocycle in bR, a blue shifted M intermediate is observed. Upon rise of the M intermediate, a proton release to the bulk solution is associated. Future time-resolved FT-IR step scan and rapid scan experiments will provide a primary structural insight into XeR´s pumping process.

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## Poster Presentations

### P-147. Interaction of synthetic polymers with biological nanopores: effects of polymer architecture and monomer size

Monasadat Talarimoghari[1], Guillaume Fiers[2], Gerhard Baaken[3], Ralf Hanselmann[4,5], Jean-François Lutz[2], Jan C. Behrends[1,5,6]

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[2]Institut Charles Sadron UPR22, Université de Strasbourg, CNRS, Strasbourg, France
[3]Ionera Technologies GmbH, Freiburg, Germany
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[5]Freiburg Materials Research Centre, Freiburg, Germany
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Biological nanopores are known to interact with synthetic and biological polymers; this property enables their use in analytical applications such as sequencing and size/mass discrimination. Our specific aim is to understand the physicochemical origin of size/mass discrimination on the basis of the depth of block, which affords a resolution better than a single monomer in the case of poly (ethyleneglycol) (PEG). To this end, we studied the interaction with α-hemolysin (αHL) and aerolysin (AL) pores of different types of synthetic polymers. To gain insight into the importance of polymer structure for this size-sensitive interaction, we compared linear and star-shaped polyethylene glycol (PEG) with the same number average molecular weight (Mn=1000 g/mol). Our results, which include a direct comparison with MALDI-TOF spectra, clearly indicate that star-shape architecture does not fundamentally alter mass-sensitivity, but clearly affects the interaction in more subtle ways. While the depth of block is slightly increased for the star-shaped architecture with respect to linear PEG of equal degree of polymerization, which may be expected from contribution of the of the glycerol core, we also observed an increased duration of the blocked levels and a lower variance of the blocked current for stars as compared to linear PEGs, suggesting a higher stability of the polymer-pore complex in the case of stars. In order to determine the molecular properties governing the depth of block during polymer pore interaction, we are in the process of studying a number of polymers with higher monomer masses than PEG. In particular, we have been able to obtain a good correlation between nanopore results and electrospray ionization mass spectrometry for data for polymers consisting of repeat units of a triazole linked to an amide group by a tri (ethyleneglycol) with monomer weight 368.2 g/mol.

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### P-148. Membrane-conditioned dimerization of G protein coupled receptors as dynamic regulation of receptor function

Stefan Gahbauer[1], Kristyna Pluhackova[2,1], Rainer Böckmann[1]

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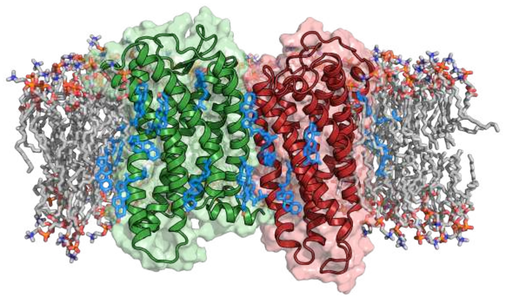
[2]ETH Zürich, Biosystems Science and Engineering, Basel, Switzerland

G protein coupled receptors (GPCRs), the largest protein family of transmembrane receptors, are essential for virtually every physiological process. Substantial evidence for GPCR dimerization indicates that receptor association regulates protein function. Furthermore, the properties and composition of the membrane environment were reported to significantly affect GPCR-mediated signaling. Chemokine receptors, a subclass of GPCRs, are essential for the immune system but also play crucial roles in cancer metastasis and HIV-infection. Receptor dimerization and cholesterol were reported to alter chemokine receptor-mediated signaling in health and disease.

Here, the dimerization of chemokine receptors in different membrane environments was investigated with extensive molecular dynamics simulations. Homodimerization of the receptor CXCR4 in phospholipid membranes was studied for increasing levels of cholesterol. Cholesterol-receptor interactions are shown to modulate dimer configurations: Cholesterol binding to the protein surface prevents the participation of certain transmembrane segments at dimer interfaces. In turn, specific dimer configurations are enabled first by cholesterol intercalation between protein monomers. In agreement with experimental studies, cholesterol-induced dimers are suggested to allow signaling activity, while dimers predominantly formed in cholesterol-free membranes likely resemble inactive structures. The closely related receptors CCR5 and CCR2 exhibit specific dimerization hotspots, distinct from CXCR4. Simulations addressing chemokine receptor heterodimerization reveal different heterodimer interfaces between CC chemokine receptors and CXCR4 entailing distinct cholesterol-induced configurational changes.

This work provides first molecular insight into the interaction network between chemokine receptors, pinpoints main dimer interfaces, and characterizes the role of cholesterol in the dynamic regulation of GPCR dimerization and function.



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### P-149. Reconstitution of Silicanin-1 into artificial lipid membranes and investigation of its self-aggregation behavior

Philipp Schwarz[1], Claudia Steinem[1,2]

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[2]Max-Planck-Institut für Dynamik und Selbstorganisation, Göttingen, Germany

Silica cell wall formation of the micro algae group called diatoms takes place in the silica deposition vesicle (SDV). Since little is known about the lipid composition of the SDV, a model membrane shall mimic the SDV membrane, called silicalemma. Solid supported membranes are a useful system to examine protein-membrane interactions and for the analysis of functional properties. Such an artificial model membrane can be used to investigate the membrane interaction with biosilica-associated molecules and its influence on silica precipitation in vitro.

Silicanin-1 (Sin1) from the diatom Thalassiosira pseudonana is a representative of the first identified transmembrane proteins which are associated with the SDV and its role for silica morphogenesis is examined. The detergent mediated reconstitution of recombinant Sin1 into artificial vesicles is followed by density gradient centrifugation and Western blotting to determine the amount of reconstituted Sin1. The change of reconstitution efficiency with varied conditions is examined. Solid supported membranes were formed by spreading Sin1 containing proteoliposomes on planar substrates. This system allows the investigation of Sin1 self-aggregation behavior with different pH values and protein-to-lipid ratios, since self-aggregation might play a role in silica morphogenesis inside the SDV.

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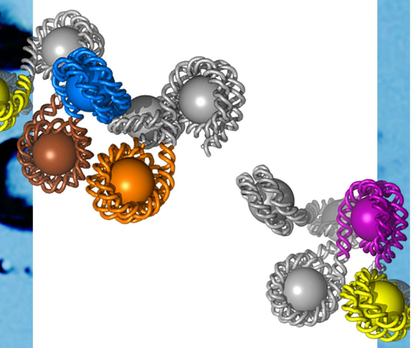



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## Poster Presentations

### P-150. Collisional lipid transfer among DIBMA-bounded nanodiscs

Bartholomäus Danielczak[1], Sandro Keller[1]

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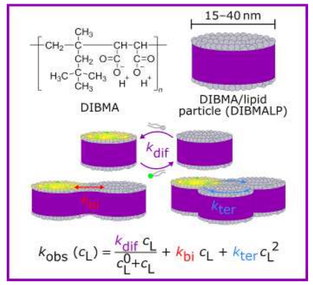
Copolymers of diisobutylene/maleic acid (DIBMA), styrene/maleic acid (SMA), and styrene/maleimide (SMI) solubilise membrane proteins and surrounding lipids directly from artificial and biological membranes to assemble into polymer-bounded nanodiscs. Although the latter preserve a lipid-bilayer core, they are much more dynamic than other membrane mimics, as we have recently demonstrated for nanodiscs bounded by SMA(3:1) and SMA(2:1). Here, we unravelled the mechanism and quantified the kinetics of lipid transfer among DIBMA-bounded nanodiscs by time-resolved Förster resonance energy transfer (FRET) spectroscopy, with particular emphasis on the roles of Coulombic repulsion and nanodisc size. Our results show that DIBMA-bounded nanodiscs exchange lipids rapidly and mostly through collisions of two or three nanodiscs. By contrast, nanodiscs bounded by SMA(3:1)- and SMA(2:1) exchange lipids considerably faster and mainly through collisions between two nanodiscs, while vesicles and nanodiscs based on membrane scaffold proteins exchange lipids much more slowly and exclusively through lipid monomer diffusion. As DIBMA is highly charged, we could accelerate collisional lipid transfer substantially by modulation of Coulombic repulsion through changes in nanodisc size and ionic strength. We rationalised the influence of the latter by applying an extended version of the Debye–Hückel equation and, thus, found an effective charge number of z = −47, which is considerably larger in magnitude than for less charged SMA(2:1)-bounded nanodiscs with z = −33.

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### P-151. Role of Coulombic repulsion in collisional lipid transfer among SMA(2:1) nanodiscs

Anne Grethen[1], David Glueck[1], Sandro Keller[1]
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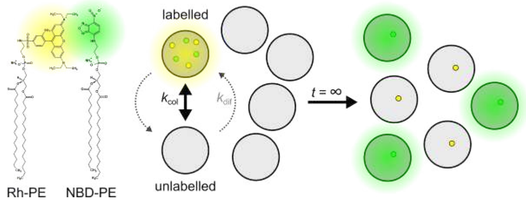
Styrene/maleic acid (SMA) copolymers are attracting great interest because they solubilise membrane proteins and lipids from native or artificial membranes to form polymer-bounded nanodiscs. These nanodiscs preserve a native-like lipid-bilayer core that is surrounded by a polymer rim and can harbour a membrane protein or a membrane-protein complex. SMA exists in various styrene/maleic acid molar ratios, which results in different charge densities, hydrophobicities, and thus, solubilisation properties. We have recently reported fast collisional lipid transfer among nanodiscs bounded by the relatively hydrophobic copolymer SMA(3:1). Herein, we employed time-resolved Förster resonance energy transfer to quantify the kinetics of lipid transfer among nanodiscs encapsulated by SMA(2:1), a less hydrophobic copolymer that is more efficient in terms of lipid and protein solubilisation. Furthermore, we assessed the role of ionic strength and, thereby, how Coulombic repulsion affects the transfer of lipid molecules among these polyanionic nanodiscs. In addition, we used zeta-potential measurements to evaluate the dependence of the effective nanodisc charge on ionic strength. Collisional lipid transfer is slower among SMA(2:1) nanodiscs (kcol = 5.9 M-1 s-1) as compared with SMA(3:1) nanodiscs (kcol = 222 M-1 s-1) but still two to three orders of magnitude faster than diffusional lipid transfer among protein-encapsulated nanodiscs or vesicles. Increasing ionic strength further accelerates lipid exchange among SMA(2:1) nanodiscs in a manner predicted by a modified form of the Debye–Hückel law that accounts for the finite size of nanodiscs. The resulting effective nanodisc charge number of z = −33 is in good agreement with the value obtained from zeta-potential measurements.

Grethen et al. J. Membr. Biol. 2018, accepted

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### P-152. Curvature dependence of SNARE TMD mediated membrane fusion

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Membrane fusion is a key event in a wide range of biological processes like exocytosis, viral infection, fertilization and intracellular trafficking. Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are thought to play a major part throughout all steps of the fusion process, e.g. docking the membranes, bringing them in close proximity by overcoming repulsive forces between the opposing membranes, initializing hydrophobic contacts, and in lowering the energy cost of highly curved membrane areas in fusion pore opening. However, the microscopic details of the individual fusion steps and especially the role of the specific domains of the SNARE proteins remain elusive.

Substituting the SNARE transmembrane domain (TMD) with lipid anchors or mutating its primary structure was shown to alter the vesicle fusion process by altering the initiation and pore opening. Molecular dynamics (MD) simulations of flat membranes were used to obtain a molecular description of these effects by investigating TMD-lipid interactions. The TMD regulates lipid mobility, and hence lipid protrusion events leading to first hydrophobic contacts between the merging membranes.

However, these studies neglect effects like changed lipid protrusion probabilities and high energy costs caused by the curvatures involved in membrane fusion. Therefore, we extend previous work on the properties of SNARE TMDs and SNARE TMD mutants using coarse-grained MD simulations allowing for strongly curved membrane geometrics.

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### P-153. Intermolecular interactions in the activation of two pore channels

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Two-pore channels (TPCs) are intracellular cation channels that are widely expressed in eukaryotic cells. In human cells and in plant cells of Arabidopsis thaliana, hTPC2 and AtTPC1 are embedded in the membrane of their acidic compartments, the lysosomes and vacuoles, respectively. Depending on the host cell, they are involved in diverse processes like cancer cell migration, Ebola virus infection, cellular cation and pH homeostasis and long-distance cell-cell signaling. The gating mechanism and regulation of these homo-dimeric channels are therefore of huge interest. It was shown that AtTPC1 gets activated upon binding of Ca2+ to the EF-hands with the second EF-hand being essential for channel activation by depolarization. Furthermore, patch-clamp experiments with wild type and truncated variants demonstrated that the C-terminus of AtTPC1 is an indispensable player for channel activity. In contrast, the homologous TPC2 of humans is gated open upon addition of phosphotidylinositols (PI(3,5)P2), however, the exact binding site and the relation to channel activation are unknown.

To investigate the mode of channel activation of AtTPC1 and hTPC2 we combined experimental patch-clamp techniques, site-directed mutagenesis and molecular dynamics simulations at the coarse-grained and atomistic level. Results demonstrated that AtTPC1 subunits interact via their C-terminal regions with a binding constant of 3.9uM for soluble C-terminal helices. In hTPC2, we successfully identified a positively charged binding pocket for PI(3,5)P2 that is formed by the S4/S5-linker and the helical extension of S6. A similar PI(3,5)P2 binding site was recently reported for the structure of another PI(3,5)P2-sensitive TPC homologue, mouse TPC1, which further strengthens our results.

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### P-154. Phospholipid order, dynamics, and hydration in polymer-bounded nanodiscs

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Lipid-bilayer nanodiscs formed from biological membranes and styrene/maleic acid (SMA) or diisobutylene/maleic acid (DIBMA) copolymers provide a native-like membrane-mimetic environment for membrane proteins. To understand the impact of the polymer on the contained lipid-bilayer patch, we investigated the order, dynamics, and hydration properties of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) within SMA/lipid particles (SMALPs) and DIBMA/lipid particles (DIBMALPs) and compared them with DMPC vesicles and micellar lipid/detergent mixtures using steady-state and time-resolved fluorescence spectroscopy. Laurdan fluorescence revealed that DIBMALPs retained lipid-packing properties similar to those in vesicles, whereas SMALPs exhibited a more rigid membrane environment. Dipolar relaxation in the lipid-headgroup region of vesicles, DIBMALPs, and SMALPs occurred on similar nanosecond timescales; however, DIBMALPs were found to be slightly more hydrated than vesicular bilayers, whereas SMALPs were the least hydrated. Time-resolved fluorescence anisotropy of 1,6-diphenylhex-1,3,5-triene (DPH) showed that membrane encapsulation by DIBMA resulted in a slight decrease in acyl-chain dynamics without any drastic impact on membrane order, which contrasted with large perturbations induced by SMA having a 3:1 styrene/maleic acid molar ratio. Taken together, our results demonstrate that DIBMALPs maintain bilayer-like lipid order, dynamics, and hydration to a greater extent than SMALPs.

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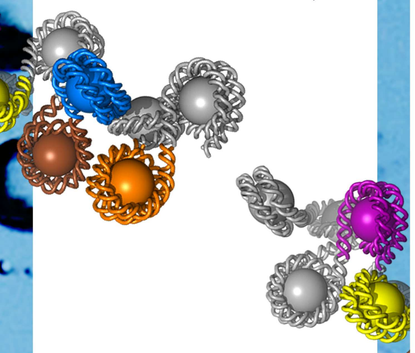



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### P-155. Phase separation of sphingomyelin contaningcontaining lipid bilayers is controlled by lipid-substrate adhesion as well as chain length and saturatio.

Jeremias Sibold[1], Vera Tewaag[1], Loan Vuong[1], Katharina Kettelhoit[2], Ingo Mey[1], Daniel B. Werz[2], Claudia Steinem[1,3]

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The lipid raft model is becoming more viable as ternary lipid mixtures containing high melting lipids (like sphingomyelin (SM)), low melting lipids (like DOPC) and cholesterol (Chol) show the coexistence of liquid disorder ($l_d$) and liquid ordered ($l_o$) phase at certain ratios of the lipids. These coexistent regions can be used to generate raft-like structures and therefore strongly suggest the phase separation. In this work we used the lipid mixture DOPC/SM/Chol (4:4:2) as an example for a phase separated membrane.

Using pore-spanning membranes (PSMs) we studied how phase separation depends on the adhesion of the lipid bilayer as it was already theoretically discussed by Lipowsky et. al[1]. They supposed that if the membrane system has adhered and non-adhered areas only one region will show phase separation, but the different areas can have different lipid compositions (phases). Phase separation and lipid composition of both areas depend on the difference in adhesion energy between the two areas.

In another project, we aim to determine how a head group labeled glycosphingomyelin is distributed in phase-separated membranes. This is analyzed by using phase separated GUVs with DOPC/$l_d$-phase marker/SM/glycosphingomyelin/Chol (39:1:39:1:20). We analyzed the influence of the saturated fatty acid length of the sphingomyelins as well as the saturation of the fatty acid of the glycosphingomyelin.

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### P-156. Permeability modes in fluctuating lipid membranes with macromolecular pores

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The study of membrane fluctuations allows access to the elastic properties of the lipid membranes. The transport of mass through pores induces additional transverse fluctuations, whose local amplitude is determined by the pore density per area. The study of large membrane pores capable of carrying macromolecular material represents an interesting model for testing the theoretical predictions that attribute active behaviours (nonequilibrium) in the permeability contributions[1]. In this work we show the experimental analysis of these active fluctuations generated from osmotic disequilibriums. To do this, we used giant unilamellar vesicles with large pores reconstituted in their membranes from the multi-protein connector of the phi29 bacteriophage packaging motor[2]. We agree with the theory by finding a dependence on the pore density in the membranes. In addition, individual permeation events have been observed in the fluctuation time-series, from which a stochastic distribution of the permeation events compatible with a shooting noise is derived.

Acknowledgements The financial support by German Research Foundation (DFG)

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### P-157. Modulation of selectivity filter gating an conductivity by the pore helix in a viral $K^+$channel

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With only 82 aa per monomer, the viral potassium channel $Kcv_{NH}$ is one of the smallest and most primitive potassium channels, which represents the pore region of more complex potassium channels and is therefore a good model. The mutant $Kcv_{NH}$ S77G is an extremely slow outward rectifier, and previous work (1) has located this gating process in the selectivity filter.

To further study the role of the selectivity filter for rectification, mutations of the Serin at position 42 in the pore helix were introduced into $Kcv_{NH}$ S77G. In KcsA the analogous position (E71) is a central element of an H-bond-network, which stabilizes the selectivity filter (2).

The mutants were electrophysiologically characterized in planar lipid bilayers. The mutations S42V and S42T both converted the channel into an inward rectifier with a threefold larger single-channel conductance. Despite the change in conductance, both mutants were is still selective for potassium.

Dwell-time analysis showed that $Kcv_{NH}$ S77G S42V and S77G S42T exhibit three different closed states with different probabilities of appearance. The reason for the low open probability at positive voltages can be reduced to one long-lived closed state with a mean dwell time of approximately 1 s.

The results show that Serine 42 is modulating the gating and conductance of the selectivity filter without affecting selectivity.

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## Poster Presentations

### P-158. Investigation of biomimetic membrane interactions with the ENTH domain of epsin 1 and substrate surfaces

Nelli Teske[1], Jeremias Sibold[1], Benjamin Kroppen[2], Johannes Schumacher[1], Martin Gleisner[1], Nikolas K. Teiwes[1], Ingo Mey[1], Michael Meinecke[2,3], Claudia Steinem[1]

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Biomimetic membrane models such as giant unilamellar vesicles (GUVs), pore-spanning membranes (PSMs) and solid-supported membranes (SSM) are well established for the investigation of structural and functional protein properties as well as membrane-protein interactions. Within these models different characteristics like lipid composition, enclosed compartment volume or membrane tension can be varied to elucidate on specific interactions. While adhered GUVs (aGUVs) offer large enclosed compartments, huge membrane areas and allow the tuning of membrane tension, SSMs enable electrical and surface-analytical methods with a high mechanical stability. If the advantages of both, solid supported and free-standing membrane areas with decreased compartment volume and free standing membrane areas are necessary, PSMs are the model of choice.

Employing aGUVs, we were able to study the effects of the epsin N-terminal homology (ENTH), a protein domain involved in the clathrin-mediated endocytosis. Treatment of aGUVs with ENTH results in changes of membrane shape due to specific receptor lipid binding and protein insertion driven by osmotic pressure and membrane tension, whereas the use of SSMs facilitate atomic force microscopy measurements to investigate the amount of ENTH binding as well as the protein height.

PSMs are better suited to study diffusion barriers and protein clustering of ENTH-membrane interactions. To overcome the major limitation of PSMs, the absent signal on the pore rims, we changed the surface chemistry resulting in low membrane tension and observable fluorescence on the whole PSM

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## Poster Presentations

### P-159. Asymmetric receptor interaction within signalosomes for the switch of canonical to noncanonical Wnt signaling

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Wnt signaling pathway is a highly conserved machinery controlling cell fate and organogenesis during embryonic development. The switch from canonical to noncanonical Wnt signaling has shown distinctive effects on stem cell differentiation and cell ageing. Upon stimulation, the transmembrane receptors and cytosolic effector proteins assemble at the plasma membrane into large multi-protein entities referred to as Wnt signalosome. The molecular and cellular principles that control the activation of canonical and noncanonical signaling by different Wnt ligands remains largely elusive. Using multicolor single molecule fluorescence imaging and life cell micropatterning, we identified that clustering of cytosolic scaffold protein Axin1 guides receptor reorganization in the plasma membrane via effector protein Dishevelled. For either canonical Wnt/β-catenin signaling or noncanonical Fzd/Ror signaling, different Wnts stimulates the ligand-specific receptor interactions within the joint signalosomes. In contrast to a neutral competition of Lrp6 and Ror2 for binding with the common receptor Frizzled, canonical Wnt signal triggers recruitment of co-receptor Ror2 for noncanonical Wnt signaling but not vice versa. These results pinpoint a critical role of liquid phase separation of cytosolic Axin1 in signalosome formation at the plasma membrane. The asymmetrical receptor interactions within Wnt signalosomes reveal a molecular machinery underlying the unidirectional switch from canonical to noncanonical Wnt signaling.

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## Poster Presentations

### P-160. Voltage-gated ion channels selective for protons

Thomas K. Berger[1], Therese Wolfenstetter[1], Lea Wobig[1], Wolfgang Bönigk[1], Heinz G. Körschen[1], U. Benjamin Kaupp[1], Reinhard Seifert[1]

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Voltage-gated ion channels have a stereotypic and modular architecture with 6 transmembrane helices (S1-S6). Helices S1-S4 form a voltage-sensor domain (VSD), and helices S5 and S6 form the pore domain (PD). A remarkable exception is the Hv1 proton channel, which contains only a VSD but lacks the PD. The VSD of Hv1 specialized so that it contains the voltage sensor, the gate, and a highly selevtive proton permeation pathway. Here, we report the discovery of a new class of hyperpolarization-activated channels, HCN-like1 (HCNL1) channels, which are highly selective for protons. Electrophysiological characterization of mutant HCNL1 channels suggests that the permeation pathway for protons lies in the VSD and not in the PD. We compare the mechanism of proton conduction in HCNL1 channels with Hv1 and discuss potential physiological roles of HCNL1 in zebrafish sperm.

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## Poster Presentations

### P-161. Free and chaperone-bound unfolded states of outer-membrane phospholipase A

Neharika Chamachi[1], Georg Krainer[1,2], Pablo Gracia[1], Erik Frotscher[2], Andreas Hartmann[1], Philip Groeger[1], Sandro Keller[2], Michael Schlierf[1]

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Structural and dynamic investigations of unfolded proteins are important for understanding protein-folding mechanisms as well as the interactions of unfolded polypeptide chains with other cell components. In the case of outer-membrane proteins (OMPs), unfolded-state properties are of physiological relevance, because they remain unfolded for extended periods of time during their biogenesis and rely on interactions with periplasmic chaperones to prevent aggregation and support correct folding. Here, we study the unfolded-state properties of outer-membrane phospholipase A (OmpLA) both in its free and chaperone-bound states. Using a combination of ensemble and single-molecule spectroscopy techniques including single-molecule FRET, we find that under strongly denaturing conditions and in the absence of chaperones, OmpLA populates an ensemble of slowly (>100 ms) interconverting and conformationally heterogeneous unfolded states that lack the fast chain reconfiguration motions expected for an unstructured, fully unfolded chain. Interestingly, when complexed with periplasmic chaperones seventeen kilodalton protein (Skp) and survival factor A (SurA), OmpLA adopts an expanded, yet squished conformational ensemble, in which the overall broad distribution of unfolded states is preserved. These findings could indicate that periplasmic chaperones Skp and SurA prestructure OMPs for their next binding partner (e.g., the β-barrel assembly machinery (BAM)) to facilitate insertion and proper folding into the outer membrane.

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## Poster Presentations

### P-162. Rationalizing the design principles of amphiphilic helical antimicrobial peptides

Erik Strandberg[1], David Benz[2], Ariadna Grau-Campistany[3], Parvesh Wadhwani[1], Jochen Bürck[1], Francesc Rabanal[3], Anne S. Ulrich[1,2]

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Antimicrobial peptides (AMPs), also known as host defense peptides, can kill microorganisms and constitute a potential new class of antibiotics. It has been proposed that some AMPs form pores in bacterial membranes, thereby destroying the cell integrity. KIA peptides are cationic amphipathic α-helical AMPs based on the sequence KIAGKIA, with varying lengths from 14 to 28 amino acids. From antibacterial assays it was found that a minimum length of KIA peptides was needed to kill bacteria, which depends on the type of bacteria. From vesicle leakage assays this length was identified as the membrane hydrophobic thickness; peptides must be long enough to span the membrane to induce leakage.

Using solid-state NMR, the orientation of the peptides in a membrane could be determined, and stable pores, formed with peptides in a transmembrane orientation, were observed in lyso-lipid containing membranes. It was further found that the activity depends on the distribution of charged and hydrophobic residues along the peptide sequence. For the short KIA14 peptide, we found that the presence of hydrophobic Ile residues are essential for function. When one of the four Ile residues was replaced by Ala, the activity was lost and the helicity was strongly reduced. Using a combination of circular dichroism, which gives global information about helicity, and solid-state NMR, giving local information, fraying of the helix was observed near the N-terminus, but not the C-terminus, and the effect of Ile on fraying was investigated.

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## Poster Presentations

### P-163. Biophysical characterization of human γ-secretase subunit presenilin and nicastrin

Ge Yang[1], Kun Yu[1], Christina-Symina Kaitatzi [2], Abhilasha Singh [1], Jörg Labahn [1]

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Human gamma secretase, a membrane protease complex comprising presenilin 1 (PS1), PEN-2, APH-1 and nicastrin. This study includes the biophysical characterization of human γ-secretase subunit presenilin and nicastrin.

Presenilin (PS1 or PS2) functions as the catalytic subunit of γ-secretase, which produces the toxic amyloid beta peptides in Alzheimer"s disease (AD). The dependence of folding and structural stability of PSs on the lipophilic environment and mutation were investigated by far UV CD spectroscopy. The secondary structure content and stability of PS2 depended on the lipophilic environment. PS2 undergoes a temperature-dependent structural transition from α-helical to β-structure at 331 K. The restructured protein formed structures which tested positive in spectroscopic amyloid fibrils assays. The AD mutant PS1L266F, PS1L424V and PS1ΔE9 displayed reduced stability which supports a proposed "loss of function" mechanism of AD based on protein instability. The exon 9 coded sequence in the inhibitory loop of the zymogen was found to be required for the modulation of the thermal stability of PS1 by the lipophilic environment.

Nicastrin, the largest member among the four components of the γ-secretase complex, has been identified to be the substrate recognizer for the proteolytic activity of the complex. Here we report that full-length human nicastrin (hNCT) can be obtained by heterologous expression in E. coli. Milligram quantities of the target protein are purified in a two-step purification protocol using affinity chromatography followed by SEC. The FOS-choline 14 purified tetrameric hNCT exhibits a proper folding with 31% α-helix and 23% β-sheet content. Thermal stability studies reveal stable secondary and tertiary structure of the detergent purified hNCT. A physical interaction between nicastrin and the γ-secretase substrate APPC100 confirmed the functionality of hNCT as a substrate recognizer.

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### P-164. Lipid selectivity in antimicrobial membrane permeabilization

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Synthetic mimics of antimicrobial peptides (smAMPs) are promising alternatives to classical antibiotics because they are less prone to resistance. Like natural antimicrobial peptides (AMPs), smAMPs are thought to act on the cell membrane, but offer the advantage of better stability and easier production. Common to all antimicrobial treatment is the need for selectivity.A series of short polymeric smAMPs with antibacterial gram-selectivity is discussed exemplarily. Advanced analysis of vesicle leakage mechanisms and kinetics reveals how the combination of smAMP design and lipid mixture can determine the mode of action. Activity and selectivity relate to molecular mechanisms like asymmetry stress, stabilization of local curvature (as in toroidal pores), and additional strong leakage events (possibly involving lipid clustering or oligomeric pores). We find antimicrobials acting by asymmetry stress less selective and more hemolytic. Conversely, an appropriate balance of electrostatically and hydrophobically mediated smAMP binding to membranes is crucial for selectivity and allows for stabilization of local curvature (e.g. in disordered toroidal pores). Selectivity can be increased by additional rare and strong leakage events such as oligomeric pores or leakage related to electrostatic lipid clustering. In order to properly infer conclusions from models to actual microbes or cells, I will discuss the relevant aspects of leakage and mention how to correct vesicle leakage experiments. A systematic view on membrane permeabilization and antimicrobial activity and selectivity holds great potential to aid future design of antimicrobials and improve membrane models for mechanistic studies.

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### P-165. Membrane budding and fission induced by adsorbing particles

Rikhia Ghosh[1], Andrea Grafmueller[1], Reinhard Lipowsky[1]
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Membrane budding and fission, which produce new membrane compartments, are essential cellular processes during organelle division, cytokinesis, membrane repair and trafficking as well as endocytosis. Fission of cellular membranes is usually executed by proteins with special structural features. Using coarse grained molecular dynamics simulations, we observe spontaneous budding and fission of a nanovesicle in a solution of small solute particles which are completely miscible with water and adsorb onto the membranes. We start from a spherical vesicle that is exposed to a very low particle concentration in the exterior solution. When we reduce the volume of this vesicle, we first observe the formation of a membrane bud which is connected to the mother membrane by a narrow neck. Even for these relatively small particle concentrations, the budding process leads to membrane fission provided the particles are strongly adsorbed.The fission process can be understood in terms of an effective constriction force that is generated by the membrane's spontaneous curvature arising from the particle adsorption.
This force is enhanced by the increased particle adsorption in the vicinity of the membrane neck. For solute particles that adsorb less strongly to the membrane, membrane budding and neck formation occur only at high particle concentration and do not lead to fission. Our results reveal a generic fission process induced by adsorption of small molecules from the aqueous solution, irrespective of the internal structure of the particles.

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### P-166. Probing the pore diameter of viral $K^+$ channels with quaternary ammonium ions

Tobias Gabriel[1], Tobias Winterstein[1], Indra Schröder[1]

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Kcv channels are small viral proteins that share strong homologies with eukaryotic and bacterial potassium channels, consisting only of the conserved pore module. With their many isoforms, they are a good model system to study structure-function correlates in $K^+$ channels. A straightforward way to investigate the structure of the cytosolic gate potassium channels via functional experiments is probing them with quaternary ammonium ions (QAs) of different size and hydrophobicity[1]. QAs are able to enter the pore from the cytosol and block $K^+$ channels by binding in the cavity directly adjacent to the selectivity filter[2].
Here, we present single-channel recordings of in vitro expressed Kcv channels in planar lipid bilayers with QAs ranging in diameter from 7 – 14 Å. Cytosolic QAs cause a voltage-dependent block. With increasing diameter and hydrophobicity, the blocking kinetics becomes slower and the affinity increases. The dependence of the binding rate constant ($k_{on}$) on the size of the blocker provides the effective diameter (8-10 Å) of the pore between the cytosol and the selectivity filter; $k_{off}$ provides a measure for the binding energy. Interestingly, TEA causes a slow as well as a fast block, while all other blockers cause either a slow or a fast block, but never both. This indicates two distinct binding sites for TEA.

[1]Jara-Oseguera et al. (2008), JGP 132:547-562
[2]Lenaeus et al. (2014), Biochemistry 53:5365-5373

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### P-167. Investigation of protein-protein and protein-lipid interactions involved in Influenza A virus assembly

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Influenza is a prominent cause of mortality in modern society and a major burden on health systems globally. The Matrix Protein 1 (M1) is an essential component involved in the structural stability of the Influenza A virus (IAV) and in the budding of new virions from infected cells. During virus assembly, M1 is recruited to the host plasma membrane (PM) where it interacts with specific lipids and other viral proteins. The structure of M1 is only partially characterized and the molecular mechanisms determining how the protein interacts with the PM, as well as those governing protein-protein interaction and multimerization, have not been yet clarified. We quantitatively investigate M1 multimerization and its interaction with lipids, both in model membranes and in living cells. To this aim, we use a combination of biophysical techniques including FRET, confocal microscopy imaging, raster image correlation spectroscopy, CD spectroscopy, surface plasmon resonance, scanning fluorescence correlation spectroscopy (sFCS) and Number and Brightness (N&B) analysis. Our results show that M1 forms multimers upon interaction with phosphatidylserine (PS)-rich domains in the PM. Protein-lipid interactions are mediated by specific residues in the N-terminal domain of M1 and cause alterations in protein structure and intra-molecular dynamics. Our experimental findings are supported by molecular dynamics simulations as monomer in solution or bound to a negatively-charged lipid bilayer. Taken together, our results provide novel quantitative information regarding the molecular interactions between IAV and host cellular membranes.

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### P-168. Light-induced budding and fission of giant vesicles

Jan Steinkühler[1], Solveig Bartelt[2], Seraphine Wegner[2], Rumiana Dimova[1], Reinhard Lipowsky[1]
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[2]2Max Planck Institute for Polymer Research, Mainz, Germany

To mimic cellular divison by synthetic proto-cells, we view the division process as a sequence of different cell shapes. Usually the starting morphology is nearly spherical. To distribute the cellular components needed to produce two daughter cells, it is necessary to form shapes of lower symmetry. Typically prolate shapes are observed. To complete the division process, two smaller spheres connected by a narrow membrane neck are formed from this prolate. Finally, the neck undergoes scission and the two spheres are separated. Apart from the scission step, all shapes have been predicted for lipid vesicles (Seifert et al, 1991). These shapes are determined by only two dimensionless parameters, the volume-to-area ratio and the spontaneous curvature. Control over membrane spontaneous curvature can be achieved by the adsorption of proteins to one membrane leaflet of a giant unilamellar vesicle (GUV). The volume-to-area ratio can be controlled by the osmotic conditions. To develop such a model system for cell division, GUVs were trapped inside dead-end channels on microfluidic chips, which allowed for the controlled exchange of the outside solution and for the observation of relaxed vesicle shapes. Initially, the experiments started with symmetric buffer conditions and spherical vesicles shapes. Osmotic deflation leads to shapes with a reduced volume-to-area ratio. Upon addition of the model protein GFP, vesicles underwent a transition to nearly symmetric dumbbell vesicles connected by a membrane neck. Within minutes, the vesicles spontaneously separated into two closed and spherical GUVs, presumably by neck scission via the effective constriction force generated by spontaneous curvature, in analogy to the force generated by adhesion (Agudo-Canalejo and Lipowsky, 2016). As the next step, we aim to replace the GFP model proteins by light induced adsorption of proteins from solutions (Bartlet et al, 2018).

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### P-169. Small molecule permeation across membrane channels: Chemical modification to quantify transport across OmpF

Mathias Winterhalter[1], Jiajun Wang[1], Jayesh Arun Bafna[1], Satya Pratyusha Bhamidimarri[1]
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Biological channels facilitate exchange of small molecules across membranes but surprisingly there is a lack of general tools for the quantification of transport. Here we use the example of OmpF, an outer membrane channel from E. coli, to introduce a molecular counter at the exit. To quantify permeation, we compare the residence time in absence and presence of an additional barrier at the periplasmic side of the porin to sense the molecules reaching the exit. Inspection of the structure suggests the aspartic acid at position 181 located below the constriction region (CR) to be suited and then we subsequently mutated this residue into cysteine in an otherwise cysteine-free OmpF and functionalzed it with 2-sulfonatoethyl methanethiosulfonate (MTSES) or the somewhat larger glutathione (GLT). Using the dwell time as the signal for translocation, we found both mono-arginine and tri-arginine permeation being reduced, while the larger sized modification drastically decreased the permeation of mono-arginine and even blocked completely the pathway of tri-arginine. In case of hepta-arginine as substrate, both chemical modifications lead to complete blocking at low voltages as observed by ion current fluctuation of the OmpFwt. As an example for antibiotic permeation, we analyzed norfloxacin. The modification of OmpF by MTSES builds a barrier sufficient to block the pathway of norfloxacin. The modulation of the residence time revealed from ion current fluctuations allows us to conclude on successful permeation of norfloxacin across OmpFwt. This approach allows to discriminate blockage from translocation events for a wide range of substrates. A potential application could be screening for permeation scaffold needed to improve the permeability of antibiotics.

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### P-170. Mechanical properties of vesicle membranes under asymmetric buffer conditions

Marzieh Karimi[1], Jan Steinkühler[1], Debjit Roy[1], Rumiana Dimova[1], Reinhard Lipowsky[1]
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Biological membranes consist of molecular bilayers which are intrinsically asymmetric in nature. This asymmetry can be induced not only by leaflet composition and specific adsorption but also by differences in the cytosolic and periplasmic solutions containing macromolecules and ions. Membranes are surrounded by aqueous buffers inside and outside the cell exhibiting strong concentration asymmetry of e.g. sodium, potassium and chlorine ions. There has been a long quest to understand the effect of these ions on the physical and morphological properties of membranes. Ion-lipid interactions and, in particular, the effect of ion trans-membrane asymmetry are crucial not only for the membrane phase state [Kubsch et al. Biophys. J. 110:2581–2584, 2016] but also influence the mechanical properties of membranes. Here, we set to explore the changes in the mechanical properties of membranes exposed to asymmetric buffer conditions. As a model membrane, we employed giant unilamellar vesicles (GUVs) and first improved existing protocols for generating GUVs in physiologically relevant salt concentrations. To assess the membrane mechanical properties, we aspirate a GUV into a micropipette and by means of an attached bead manipulated via optical tweezers, we pull an outward tube to measure the spontaneous curvature and the bending rigidity of the bilayer. With increasing the aspiration pressure, the bead is displaced from the equilibrium position in the optical trap, which in return gives us the bending rigidity and spontaneous curvature of GUVs [Lipowsky, Faraday Discuss. 161:305-331, 2013]. We explore the effect of asymmetric distribution of salt and sugars across the membrane.

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

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## Poster Presentations

### P-171. Spin-labeled nanobodies: a new tool towards Electron Paramagnetic Resonance (EPR) studies in cellular environments.

Laura Galazzo[1], M. Hadi Timachi[1], Gianmarco Meier[2], Cedric A.J Hutter[2], Markus A. Seeger[2], Enrica Bordignon[1]

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[2]University of Zurich, Institute of Medical Microbiology, Zürich, Switzerland

ATP-binding cassette (ABC) transporters pump substrates across the membrane by coupling ATP-driven movements of nucleotide-binding domains to the transmembrane domains, triggering the switch between inward- and outward-facing (IF and OF) conformations. Among others, these proteins translocate lipophilic compounds, leading to multidrug resistance of cancer cells. In this framework, site-directed spin labeling EPR in combination with DEER (Double Electron Electron Resonance) are powerful techniques to monitor the large-scale conformational transition of this class of membrane proteins, necessary to understand the mechanisms of substrate translocation.

Besides spin labeling engineered cysteines in the transporter, here we present the use of spin-labeled nanobodies specifically designed to target two distinct ABC exporters as conformational reporters.

With the use of a synthetic nanobody we unraveled activity-related structural details of the conformational equilibrium of the heterodimeric exporter TM287/288 using spectroscopically orthogonal nitroxide and gadolinium (Gd) labels. Additionally, this nanobody aided the crystallization of the OF state of TM287/288, by specifically binding to the extracellular region of this state. On the other hand, we investigated the use of a natural nanobody which targets the nucleotide binding domains of the homodimeric exporter MsbA without affecting its ATPase activity. The presence of two nitroxide- or Gd-labeled nanobodies per MsbA dimer during the whole nucleotide cycle allowed to indirectly monitor the conformational cycle of the wild type unlabeled transporter through detection of inter-nanobody distances. This paves the way for the use of Gd-labeled nanobodies as conformational reporters of MsbA in cells, in order to tackle the still unsolved questions regarding its conformational plasticity in a native environment. Regarding this breakthrough in the field, our first results in inside-out E. coli vesicles will be presented.

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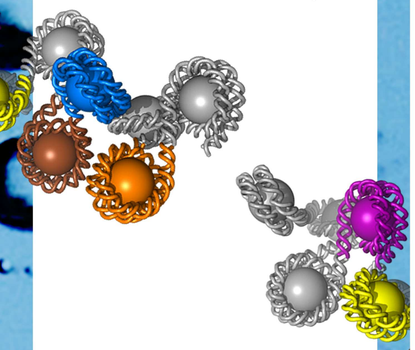



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### P-172. Supramolecular host-guest systems for biomembrane transport of peptides

Andreas Hennig[1]

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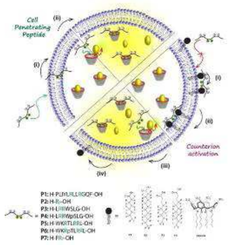
The possiblity to combine supramolecular recognition systems based on host-guest chemistry with biomolecules presents an interesting endeavor with endless possiblities, for example, to facilitate membrane transport and ultimately cellular uptake of otherwise impermeable molecules or to afford sensor systems for monitoring membrane transport. We have recently shown that amphiphilic calixarenes are excellent counterion activators for cell-penetrating peptides (CPPs), which can even transport otherwise membrane-impermeable peptides across phospholipid bilayer membranes of liposomes depending on the phosphorylation state of the peptides
(Angew. Chem. Int. Ed. 2017, 56, 15742).

We now report that supramolecular reporter pairs, which we have used for following enzyme activity (e.g. Chem. Eur. J. 2012, 18, 3444 and Angew. Chem. Int. Ed. 2015, 54, 13444), can be used for following membrane transport of unlabeled cell-penetrating peptides in large and giant unilamellar vesicles by fluorescence spectroscopy and we use this supramolecular membrane transport assay to investigate the mechanism of our membrane-transporting amphiphilic calixarenes in more detail.

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## Poster Presentations

### P-173. Green light-sensing in the corn smut fungus Ustilago maydis: biophysical characterization of the fungal rhodopsins UmOps1 and UmOps2

Ulrich Terpitz[1], Sabine Panzer[1], Annika Brych[2], Alfred Batschauer[2]

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[2]Philipps-University, Department of Plant Physiology and Photobiology, Marburg, Germany

In many fungi, green light is sensed by microbial rhodopsins, proteins that consist of seven transmembrane domains (TMDs) and show structural and functional similarity with bacteriorhodopsin. In these proteins, the chromophore all-trans-retinal is covalently bound via a Schiff" base to a lysine residue in the seventh TMD. Although rhodopsins are widespread in the fungal kingdom, today only few of them are electrophysiologically characterized and little is known about their biological role. This is remarkable, as recent findings suggest a role of rhodopsins in the colonization of plants in phytopathogenic and -associated fungi [1]. The fungal pathogen Ustilago maydis infects corn plants, thereby provoking severe tumours on all aboveground parts of the host plant. In U. maydis three genes encoding for putative photoactive opsins, called Umops1 (UMAG_02629), Umops2 (UMAG_00371), and Umops3 (UMAG_04125), are found. UmOps1 and UmOps2 are also expressed in absence of the host, whereas UmOps3, recently annotated as a heat shock protein, is only expressed during plant infection [2]. Here we show by patch-clamp analysis that UmOps1 and UmOps2 are green-light driven, outward-rectifying proton pumps. UmOps1 exhibits an extraordinary pH dependency and its pumping activity is strongly augmented by weak organic acids (WOAs), especially by the auxin indole-3-acetic acid (IAA). In contrast, UmOps2 shows the typical characteristics of a proton pump and no response to WOAs. As shown by 3D structured illumination microscopy of U. maydis sporidia, UmOps1 is mainly localized in the plasma membrane, whereas UmOps2 is localized in vacuole membranes. If and how the rhodopsins interact within the fungus to fulfil their biological roles will be task of future investigations. This is the first functional characterization of fungal rhodopsins from basidiomycetes.

References
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[2] Gosh, A., 2014, FEMS Microbiology Letters 361 (1): 17–24

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### P-174. Do the signaling proteins N-Ras and K-Ras4B colocalize in model biomembranes?

Lei Li[1], Mridula Dwivedi[1], Nelli Erwin[1], Simone Möbitz[1], Roland Winter[1]
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N-Ras and K-Ras4B are oncoproteins and play a pivotal role in cellular signalling pathways. In vivo studies suggest that intermolecular interactions foster the self-association of both N-Ras and K-Ras4B and the formation of nanoclusters in cell membranes. As sole sites for effector binding, nanocluster formation is thought to be essential for signal transmission of both N-Ras and K-Ras4B. It is reported that a lipid-mediated effect may induce a crosstalk between spatially segregated H-Ras and K-Ras4B. Further, GTP-bounded over-expression of H-Ras has been shown to even disrupt K-Ras4B clustering [(1)]. The mechanism of the crosstalk between spatially segregated H-Ras and K-Ras4B in cell membranes is still unknown. Previous in vitro studies reported that N-Ras and K-Ras4B both localize in the disordered phase of heterogeneous model membranes [(2)]. However, the simultaneous localization of N-Ras and K-Ras4B and subsequent changes in lateral organization of the membranes has not been studied, yet. In this study, we revealed that N-Ras and K-Ras4B localize adjacent (<10 nm) in the disordered phase of heterogeneous membranes using Förster resonance energy transfer (FRET) methodology. The N-Ras and K-Ras4B proteins do not colocalize in the lipid bilayer, however. Furthermore, we found that N-Ras and K-Ras4B diffuse independently in the buffer surrounding the lipid membranes.

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## Poster Presentations

### P-175. Catch me if you can: microfluidic traps for manipulating and studying active processes in GUVs

Kristina Ganzinger[1], Nils Chapin[1], Michael Heymann[1], Petra Schwille[1]
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Cell-sized giant unilamellar vesicles (GUVs) from natural or non-natural lipids are widely used as a model system for cellular membranes. With the goal of an ever-better understanding of processes at biomembranes, researchers reconstitute increasingly complex biomimetic systems in GUVs. However, it is still not trivial to generate GUVs with an asymmetrically functionalised membrane, often a basic requirement for biological mimicry. In particular, electroformation creates symmetric GUVs by default, requiring subsequent introduction of asymmetry which is hampered by their fragility. Here, we present a microfluidic chip for entrapment of GUVs in 1mm-wide channels using PDMS posts. Our design of post positioning maximises trapping efficiency, so even for GUV preparations with low yields high-quality GUVs can be readily observed. On-chip capture of GUVs allows to control the composition of the external solution, thereby enabling detachment of proteins specifically from the outer GUV membrane leaflet. Unspecific GUV and protein adhesion to the traps was prevented by PDMS coating. Our improved microfluidic traps provide an efficient tool for GUV manipulation during microscopic observation, increasing through-put and opening new avenues for experimental studies of biomolecules in GUVs.

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### P-176. Influence of the osmolyte TMAO on the thermo- and barotropic phase behavior of lipids

Magiliny Manisegaran[1], Irena Kiesel[1], Mimi Gao[2], Roland Winter[1]

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Biological cells use compatible osmolytes against various environmental stress factors. Generally, stress factors include low and high temperature, desiccation, shear, and osmotic pressure. Trimethylamine-N-oxide (TMAO) is an osmolyte known also for its pronounced stabilizing effect on proteins against high-hydrostatic pressure stress in deep sea fish. Besides the stabilization against pressure, it protects proteins also against denaturation at high temperatures or destabilizing agents such as urea. Little is known about the effect of TMAO on other biomolecular systems, such as lipid membranes. Here, we present results on the influence of TMAO on two lipid systems, a one-component phospholipid bilayer system (DMPC) and a heterogeneous three-component lipid system (DOPC:DPPC:Cholesterol 1:2:1). The structure of the lipid membranes and their phase behavior have been studied over a wide range of temperatures and pressures as well as TMAO concentrations applying FTIR, fluorescence spectroscopy and small-angle X-ray scattering (SAXS). The results obtained allowed us to reveal TMAO-induced changes in supramolecular structure, hydration level as well as the phase behavior of the model biomembrane systems.

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### P-177. Single-molecule FRET spectroscopy of membrane-protein folding structural dynamics and thermodynamics

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Membrane proteins fulfill a plethora of vital functions, are major drug targets, and are implicated in many diseases. However, their relevance is in no way paralleled by our understanding of the processes by which these proteins fold into cellular membranes. Current methods used in the study of membrane-protein folding are often limited in their ability to resolve the structural, thermodynamic, and kinetic details of folding reactions within lipid-bilayer membranes or membrane-mimetic environments. Here we developed single-molecule Förster resonance energy transfer (FRET) spectroscopy as a tool to study the spatial, temporal, and energetic features of membrane-protein folding landscapes. Leveraging the capabilities of a multiparameter single-molecule FRET and fluorescence correlation spectroscopy platform that allows for the characterization of folding dynamics spanning timescales from nanoseconds to hours, the applicability of single-molecule FRET spectroscopy to questions of membrane-protein folding dynamics was explored in three lines of research: (i) the kinetic and thermodynamic folding-landscape modulation of the α-helical membrane protein Mistic by properties of its environment, (ii) the unfolded-chain conformational dynamics of the β-barrel membrane protein Outer-membrane phospholipase A (OmpLA) both in its free and chaperone-bound state, and (iii) the structural and energetic effects of mutation-induced misfolding of the cystic fibrosis transmembrane conductance regulator (CFTR) and its drug-rescue by a chemical corrector. The versatility brought about by single-molecule FRET spectroscopy to questions of membrane-protein folding dynamics and thermodynamics offer exciting perspectives for future investigations and lay ground for this methodology to become an integral tool in solving the membrane-protein folding problem.

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### P-178. Conformational dynamics of the autophagy-related protein GABARAP on multiple time scales

Irina Apanasenko[1,2], Christina Möller[1,2], Jakub Kubiak[3], Oliver Schillinger[2,4], Ralf Kühnemuth[3], Kai Schmitz[1,2], Dennis Della Corte[5], Dieter Willbold[1,2], Birgit Strodel [2,4], Claus A. M. Seidel[3], Philipp Neudecker [1,2]

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The 117-residue $GABA_A$ receptor-associated protein (GABARAP) interacts with many different molecules to perform important regulatory functions in vesicle transport and fusion events in autophagy and apoptosis [1]. To this end, GABARAP is enzymatically lipid-conjugated to allow anchoring to autophagic membranes, which has been reported to facilitate membrane hemifusion upon oligomerization. Structure determination of GABARAP by NMR [2] and X-ray crystallography [3] suggested significant conformational heterogeneity and dynamics. Intriguingly, crystallization under high salt conditions resulted in an alternate conformation in which the N-terminal region is associated with the hydrophobic binding pockets of a neighboring molecule [3]. Unfortunately, it remains unclear whether this alternate conformation indeed facilitates oligomerization during membrane fusion and/or tubulin polymerization or is merely a crystallization artifact. The structural details, kinetics and thermodynamics as well as the functional relevance of the conformational heterogeneity are still poorly understood.

Understanding the molecular mechanisms of such a multifunctional protein as GABARAP requires knowledge not only of its tertiary structure but also of its conformational dynamics. NMR spectroscopy is a powerful tool for studying structure and dynamics on virtually all time-scales at atomic resolution. In particular, sub-nanosecond dynamics determine spin relaxation rates, whereas the biochemically often more relevant dynamics on the micro- to millisecond time-scale causes line broadening, which can be quantified by Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion (RD) experiments [4]. As a complementary technique the fluorescence spectroscopic toolkit provides insight into long-range dynamics. Thus, minor conformational states and local flexibility of proteins on the microsecond to sub-millisecond time-scale are accessible by single-molecule multiparameter fluorescence detection (MFD) [5], correlation techniques (filtered FCS) [6], and ensemble lifetime-resolved fluorescence (eTCSPC). Molecular dynamics (MD) simulations describe protein dynamics directly at atomic resolution, thereby allowing interpretation of experimental data from NMR and fluorescence spectroscopy on the structural level.

To determine the dynamics of GABARAP, we have measured 15N relaxation rates, {1H}15N heteronuclear NOEs, and 15N CPMG RD profiles, which quantify the hydrodynamics of the protein and reveal conformational dynamics in various regions of the tertiary structure. Residues in the termini and loop regions are highly mobile on the nanosecond time-scale as indicated by low order parameters and lifetime-resolved fluorescence anisotropy. CPMG RD experiments reveal two distinct conformational exchange processes on the millisecond time-scale. Specifically, resonances in the N-terminal helical subdomain exhibit separate resonances as a result of exchange on a time-scale of several milliseconds between two conformations with similar equilibrium populations. By contrast, residues lining the hydrophobic binding pockets reveal a slightly faster exchange process with an excited state population of about 1-2%. These two millisecond exchange processes are present both in cytosolic GABARAP as well as in GABARAP anchored to nanodiscs as a membrane mimetic. They are further modulated by point mutations and variations in ionic strength. Investigation of the structural details of these conformational exchange processes is tackled by CPMG RD experiments on a variety of different nuclei. In addition, filtered FCS and eTCSPC suggest large-amplitude structural rearrangements of the N-terminal part of GABARAP occurring on the microsecond time-scale. The fact that dynamics are prominent over virtually all time-scales emphasizes the importance of characterizing conformational changes for achieving a complete picture of GABARAP.

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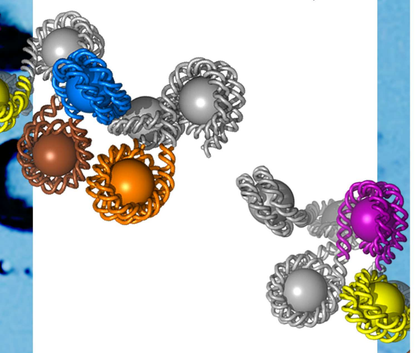



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## Poster Presentations

### P-180. FTIR-imaging of human bladder tissue for cancer biomarker research

Frederik Großerüschkamp[1], Angela Kallenbach-Thieltges[1], Kathrin Witzke[2], Melanie Horn[1], Hendrik Jütte[3], Florian Roghmann[4], Nicolas von Landenberg[4], Thilo Bracht[2], Heiko Käfferlein[5], Thomas Brüning[5], Karin Schork[2], Martin Eisenacher[2], Katrin Marcus[2], Andrea Tannapfel[3], Joachim Noldus[4], Barbara Sitek[2], Klaus Gerwert[1]

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The power of FTIR-imaging for clinical diagnosis was shown before by several other groups including us with accuracies >95%. By integration of FTIR-imaging and supervised clustering algorithms we were able to annotate automated and label free human tissues of lung, colon and bladder. Besides, we combined the imaging results with a deeper molecular understanding of the tissue for biomarker research. Therefore, we integrated the imaging results with laser capture microdissection (LCM) for collecting label free homogeneous samples for omics analysis.

In our approach the unknown tissue spectra were classified by a previously trained RF like a fingerprint. The tissue is annotatedwith high spatial accuracy. Afterwards, the annotation is transferred to a LCM system which allows the collection of label free homogeneous tissue samples. In the presented study we used label-free LC-MS to identify protein biomarkers. The identified markers were afterwards validated by immunohistochemistry (IHC).

Bladder cancer (BC) and cystitis samples of 10 vs. 7 patients were analyzed. By using the FTIR-guided LCM approach it was possible to harvest regions of pure high grade BC and cystitis with >95% sensitivity and >95% specificity – validated for over 100 patients. The proteomic results allowed us to identify over 70 differentially abundant proteins. One of these was identified as a new biomarker that is able to distinguish between cystitis and high grade carcinoma. The protein biomarker was validated in IHC for around 300 patients.

The new biomarker is able to distinguish between high-grade carcinoma and cystitis in bladder. Beside the identification of the new protein biomarker, the results will help to gain acceptance in the clinical community for FTIR/IR-imaging in tissue annotation.

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### P-181. QCL based immuno-IR sensor as Alzheimer blood test

Grischa Gerwert[1], Jörn Güldenhaupt[1], Andreas Wieck[2], Klaus Gerwert[1]
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Protein analysis with biosensors based on attenuated total reflection infrared (ATR-IR) spectroscopy is an emerging technique. It allows protein secondary structure analysis in water solutions. Using specific antibodies bound to the ATR-surface a specific biomarker can be extracted out of the biofluid. Thereby the secondary structure distribution of misfolded proteins can be determined in blood by measuring their amide I band frequency. Up to now Fourier-transform infrared (FTIR) spectroscopy was used for these ATR-based immuno-IR sensors. However, in contrast to the globar light source of the FTIR set-up quantum-cascade lasers provide a coherent high intensity beam with sub mm diameter. Here, the newest generation of quantum cascade laser (QCL) based spectrometers is used. They are very promising systems and are able to compete with FTIR regarding signal to noise ratio (SNR).

Here we present the first study with a QCL based immuno-IR sensor for detection of the folding state of monolayers of immobilized Aβ (β-Amyloid) protein bound to an antibody. Misfolding of the Aβ protein to β-sheet confirmations and their deposition is associated with Alzheimer"s disease. For the experiments we utilized the QCL-spectrometer ChemDetect (Daylight Solutions, San Diego, USA), a patented chemically modified germanium ATR crystal with attached antibodies with 25 internal reflections and a home-build automated flow-through system. For comparison we performed measurements with a Vertex 70 (Bruker, Ettlingen) spectrometer. We found that the amide I bands are of the same shape and the spectra have similar SNR. Overall, we show that the ChemDetect system can be used to perform measurements on protein monolayers for secondary structure determination. However, unlike an FTIR spectrometer, the QCL-spectrometer is about 1/12 times smaller, much less expensive, does not need liquid nitrogen for detector cooling and needs less sample material as compared to FTIR based ATR-setup.

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### P-182. Investigation of the effect of effector molecules on disease related proteins and their secondary structure via ATR-FTIR-spectroscopy

Léon Beyer[1], Julia Lange[1], Brian Budde[1], Marvin Mann[1], Andreas Nabers[1], Klaus Gerwert[1]

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**Introduction:** Alzheimer"s disease (AD) diagnosis requires invasive CSF analysis or expensive brain imaging. Therefore, a sensitive blood test is requested to reduce the number of individuals that may undergo these comprehensive follow-up examinations.

**Methods:** We applied an immuno-infrared-sensor to measure the Aß and Tau secondary structure distribution in plasma and CSF (61 controls, 39 AD cases).

**Results:** In a 2-step diagnosis, blood-plasma analyses yielded a sensitivity of 90%. Subsequently, CSF samples of individuals with indicated AD were investigated according to the Aß and Tau secondary structure distribution. Using a simple majority vote classifier, an overall specificity of 97% was observed.

**Discussion:** The 2-step AD diagnosis enables a blood-based preselection of individuals with suspected AD for a more detailed and invasive CSF analysis. Moreover, the 2-step diagnostics provide a high overall sensitivity and specificity. Thus, this approach is also of great value for the preselection of individuals for clinical studies focused on Aß.




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### P-183. 2-Step Alzheimer´s Disease Diagnosis: Plasma Aβ for preselection of Alzheimer´s individuals

Julia Lange[1], Andreas Nabers[1], Laura Perna[2], Ute Mons[2], Jonas Schartner[1], Jörn Güldenhaput[1], Kai-Uwe Saum[2], Shorena Janelidze[3], Bernd Holleczek[4], Dan Rujescu[5], Oskar Hansson[3,6], Hermann Brenner[2,7], Klaus Gerwert[1]

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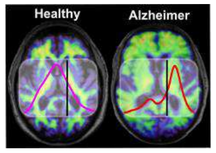
Alzheimer"s disease (AD) is currently incurable, but there is general agreement that a minimally invasive blood-biomarker for screening in preclinical stages would be crucial for future therapy. Diagnostic tools for detection of AD are either invasive like cerebrospinal fluid (CSF) biomarkers, or expensive such as Positron Emission Tomography (PET) scanning. Here, we determine the secondary structure change of Amyloid-β (Aβ) in human blood. This change used as blood amyloid biomarker indicates prodromal AD and correlates with CSF AD biomarkers and amyloid PET imaging in the cross-sectional BioFINDER cohort. In a further population-based longitudinal cohort (ESTHER) the blood-biomarker detected AD several years before clinical diagnosis in baseline samples with a positive likelihood ratio of 7.9, i.e., those who were diagnosed with AD over the years were 7.9 times more likely to test positive. This assay may open avenues for blood screening of early AD stages as a funnel for further more invasive and expensive tests.

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### P-184. Throughput optimized regenerative immuno-infrared-assay for the diagnosis of alzheimer's- and parkinson's disease

Brian Budde[1], Marvin Mann[1], Léon Beyer[1], Julia Lange[1], Dominik Röhr[1], Carsten Kötting[1], Andreas Nabers[1], Klaus Gerwert[1]

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**Introduction:** The diagnosis of Alzheimer"s disease (AD) and Parkinson"s disease (PD) are challenging and complex fields. AD is a protein misfolding disease where Amyloid beta (Aβ) and Tau undergo secondary structure change up to 20 years before clinical manifestation. The development of assays based on body fluids like blood or cerebrospinal fluid (CSF) is the subject of current research. We designed a label-free immuno-Infrared-sensor to detect changes in secondary structure distribution of Aβ and Tau present in blood and CSF.

**Methods:** The sensor uses chemically modified germanium crystals (applying NHS silanes) to covalently bind protein-specific monoclonal antibodies. To achieve higher throughput in an automated set-up, a novel approach based on a microbial immunoglobuline binding protein (Protein A) is in development. In this novel approach, Protein A is covalently attached to the sensor surface and the protein-specific antibodies bind to Protein A. This allows multiple measurements within the same system by performing multiple binding and elution cycles.

**Result:** The sensor distinguishes between monomeric and fibrillary Aβ isoforms based on the shift of the amide I band. Protein A can be attached to the sensor without losing its ability to bind antibodies. This modified sensor surface is still able to detect Aβ and Tau in complex body fluids. Elution experiments revealed that at least 7 binding cycles of antibodies on attached Protein A are possible but need to be improved and optimized.

**Discussion:** The immuno IR sensor enables a conformation-specific and label-free analysis of Aβ and Tau in complex fluids like blood and CSF. The development of a regenerative sensor chip is a crucial step to obtain an economical and time-saving assay with less user input.

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### P-185. The small molecule anle138b shows interaction with α-synuclein oligomers in phospholipid membranes

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Neurodegenerative diseases remain a major challenge to modern day society. A physiological hallmark of these diseases is the presence of deposits in the patient"s brain, which consist of various aggregates of abundant proteins such as amyloid-β (Alzheimer"s Disease) or α-synuclein (Parkinson"s Disease). Growing evidence suggests that oligomers of these proteins, formed during the aggregation process, constitute the major toxic species.
In our and collaborating groups a series of 3,5-diphenyl-pyrazole derivatives have been designed and synthesized to interfere with the processes associated with neurodegenerative diseases. Among these compounds, anle138b showed the highest efficacy in mouse models of taupathies, Parkinson"s-, Alzheimer"s- and Prion Disease. The success of anle138b in vivo calls for an elucidation of the underlying mechanism in vitro.
By a combination of NMR-, fluorescence-, and CD-spectroscopy the kinetics of α-synuclein in the presence of phospholipid membranes were thoroughly investigated. The exact knowledge of the kinetics in return allowed the enrichment of protein oligomers in the presence of lipids. Using DNP-enhanced solid-state-NMR we could find an interaction of membrane embedded anle138b with α synuclein oligomers. Further investigation of the membrane-associated oligomers was done by Atomic Force Microscopy. NOESY-spectra of the compound yielded both its location and conformation inside the lipid bilayers, which will enhance understanding of the interaction. These findings give us confidence in revealing valuable information about the mechanism of not only the disease related proteins, but also potential therapeutic targets in the aggregation process.

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### P-186. Opposed effects of dityrosine formation in soluble and aggregated α-synuclein on fibril growth

Michael Wördehoff[1], Hamed Shaykhalishahi[1], Luca Groß[1], Lothar Gremer[1,2], Matthias Stoldt[1,2], Alexander Buell[1], Dieter Willbold[1,2], Wolfgang Hoyer[1,2]

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Parkinson's disease is the second most common neurodegenerative disease. It is characterized by aggregation of the protein α-synuclein (α-syn) in Lewy bodies and increased oxidative stress in the substantia nigra. Oxidative stress leads to modifications of biomolecules, e.g. dityrosine (DiY) crosslinking in proteins, which has recently been detected in α-syn in Lewy bodies from Parkinson's disease patients. Here we report that α-syn is highly susceptible to ultraviolet (UV)-induced DiY formation. We investigated DiY formation of α-syn and nine tyrosine-to-alanine mutants and monitored its effect on α-syn fibril formation. UV irradiation of α-syn generates DiY-modified monomers and dimers, which inhibit fibril formation of unmodified α-syn by interfering with fibril elongation. The inhibition depends on the DiY group and its integration into α-syn. When preformed α-syn fibrils are crosslinked by DiY formation, they gain increased resistance to denaturation. DiY-stabilized α-syn fibrils retain their high seeding efficiency even after being exposed to denaturant concentrations that depolymerize non-crosslinked seeds. Oxidative stress-associated DiY crosslinking of α-syn therefore entails two opposing effects: (i) inhibition of aggregation by DiY-modified monomers and dimers, and (ii) stabilization of fibrillar aggregates against potential degradation, which can lead to promotion of aggregation, especially in the presence of secondary nucleation.

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### P-187. Integrative analysis of an UTX (KDM6A) interaction subset using high precision fluorescence microscopy (MFIS-FRET) guided by biochemical methods

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The H3K27 specific demethylase UTX (KDM6A) is part of a large and highly dynamic complex scaffolded around the H3K4 mono-methyltransferase MLL2 (KMT2D). UTX and MLL2 display a close functional cooperation since both H3K27 demethylation and H3K4 methylation are major gene activating factors. Although the core composition of the MLL2-complex is established, details on the interaction sites and the protein arrangement remain elusive. Flexible regions involved in interaction found in the proteins of the complex often escape structural characterization by static methods. Multiparameter Fluorescence Imaging combined with Förster Resonance Energy Transfer (MFIS-FRET) was utilized to search for structural information in the UTX interaction subset of the MLL2-complex. Biochemical methods were used to identify basic interaction while MFIS-FRET was used to validate and refine the results while adding structural information. Initial experiments were focused around a possible self-interaction of UTX and its interaction with RBBP5, a core component of the MLL2-complex which is pivotal for methyltransferase activity. Results show a promising direct interaction between UTX and RBBP5 but no self-interaction (homo-dimerization) of UTX. Subsequent analysis will address other core components of the MLL2-complex, particularly highlighting the nature of interaction between UTX and MLL2 as part of their unique functional relationship. Since somatic mutations in UTX and MLL2 genes are highly and overly abundant in urothelial carcinoma, understanding the underlying biological mechanisms could contribute to a broad range of applications for clinical science and cancer treatment.

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### P-188. Targeting acute myeloid leukemia with a small-molecule protein-protein interaction inhibitor

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Acute myeloid leukemia (AML) is a malignant disease of immature myeloid cells and it is the most prevalent acute leukemia among adults. AML is characterized by the chromosomal translocation t(8:21), which generates the oncogenic fusion protein AML1-ETO (Eight twenty one, encoded by RUNXT1). The nervy homology region 2 (NHR2) domain of ETO mediates homo-tetramerization of AML1-ETO, and this oligomerization is essential for oncogenic activity. In this study, we aimed to inhibit tetramerization of NHR2 by a high affinity and specific small-molecule inhibitor, a new therapeutic agent suppressing RUNX1/ETO oncogenic activity and, thus, exerting an anti-leukemic effect. Previously, we have identified hot spot residues that are essential for the tetramerization of NHR2, followed by the identification of the inhibitor 7.44 using structure-based virtual screening. Here, we show that the heterologously expressed NHR2 domain forms a stable tetramer as determined by analytical ultracentrifugation and size exclusion chromatography. Biophysical assays based on micro scale thermophoresis and differential scanning fluorimetry demonstrate the binding of 7.44 to NHR2, with a dissociation constant in the range of lower micromolar affinity. Furthermore, identification and mapping of the binding epitopes were achieved using saturation transfer difference (STD) and multidimensional NMR experiments. Our results suggest that 7.44 could serve as a lead structure to guide the development of structurally related compounds with increased binding affinity, improved bioavailability, and enhanced anti-leukemic effects.

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### P-189. Magnetic cell labelling: Single cell evaluation and magnetic sorting

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The magnetic labelling of cells is essential for all applications dealing with in vivo cell tracking by MRI, magnetic hyperthermia, cell separation and targeted delivery and controlled release of drugs.

In the usual experimental procedure superparamagnetic iron oxide nanoparticles (SPIO-NP) are added to the growth medium of the cells. Typical SPIO-NP concentrations are between 1-100 µg/ml and incubation times range from some hours to several days. The cytotoxicity of SPIO-NPs is normally related to the amount of nanomaterials present in the cell medium, but this information does not provide any information regarding safe concentrations of nanoparticle usage since the distribution of SPIO-NPs on cells are usually not homogeneous.

There are many different methods to determine the amount of nanoparticles per cell like Prussian blue staining, vibrating sample magnetometry (VSM), magnetic force microscopy (MFM), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and magnetic resonance (MR) relaxometry. These methods require expensive equipment and/or determine the mean amount of magnetic particles distributed over the whole cell sample. Thus it is not possible to determine the exact amount of SPIO-NPs inside single cells with high throughput and accuracy. But exactly this aspect is essential to determine the toxic effects of intracellular nanoparticles.

Here two magnetophoretic microfluidic strategies are presented: one to determine the amount of internalized SPIO-NP and one to sort the cells with respect to the amount of internalized SPIO-NP. To increase accuracy and decrease effort of evaluation a semi-automated detection and tracking strategy has been developed. The results show that the magnetic content of cells can be determined, thus this method allows highly precise high-throughput sorting according to magnetic content.

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### P-190. Nanobud formation and nanoparticle engulfment by bilayer membranes with compositional asymmetry

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We study the mechanical and curvature-elastic properties of bilayer membranes with compositional asymmetry by molecular simulations. The compositional asymmetry is controlled by the mole fraction $\phi_1$ of lipids with a bulky head group in one of the bilayer leaflets. As we increase the mole fraction $\phi_1$ of the bulky-head lipids, we observe a remarkable evolution of the stress profile across the bilayer and a strong increase in the first moment of this profile. In order to extract the spontaneous curvature from this moment, we also determine the bending rigidity of the bilayer which is found to exhibit a non-monotonic dependence on $\phi_1$. The resulting spontaneous curvature is found to be quite large compared to other molecular mechanisms for bilayer asymmetry.[1] The generated curvature leads to the formation of nanobuds, which provide new membrane compartments, in close analogy to cellular budding processes. Bilayers with compositional asymmetry can also be used to elucidate the influence of spontaneous curvature on the engulfment of nanoparticles.[2]

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[2] J. Agudo-Canalejo and R. Lipowsky: Critical Particle Sizes for the Engulfment of Nanoparticles by Membranes and Vesicles with Bilayer Asymmetry. ACS Nano {9}, 3704 (2015).

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### P-191. Probing polymer chain conformation and fibril formation of peptide conjugates

Bruno Voigt[1], Zhanna Evgrafova[2], Monika Baumann[1], Madlen Stephani[1], Wolfgang H. Binder[2], Jochen Balbach[1]

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The parathyroid hormone (PTH) is an 84 residue peptide produced by the parathyroid glands controlling the calcium and phosphate level in human blood. The peptide adopts an α-helical conformation at the N-terminus and is intrinsically disordered at the C-terminus. Amyloidogenic properties of PTH have been reported [1]. To get further insights into the mechanism of amyloid fibrillation we investigated the effect of thermoresponsive polymers on PTH [2]. We covalently attached polyacrylate based polymers to 15N isotope labelled PTH 1-84 and employed two dimensional NMR spectroscopy techniques for the characterization of the resulting chimaeras. This allows the visualization of amino acid specific changes of the peptide backbone according to the conformation of the conjugated polymer. The studies revealed strong dependencies of chemical shifts on the temperature, the peptide attachment site and the polymer molecular weight. However, conjugated PTH is still able to form amyloid fibrils though it shows altered aggregation kinetics.

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### P-192. Imaging and epidemiology of breast cancer in the region of Chlef in Algeria

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The objective of this work is to estimate some epidemiological and biostatistical parameters such as: Incidence rate, mean age and others by different methods, to determine some important risk factors and the role of the use of medical imaging to screen and diagnose cancer in the “Physics of Cancer” framework. The role of clinical laboratories in identifying the type and stages of cancer before treatment begins. To develop the local and national cancer registry. Our work concerns a retrospective series of 838 cases of breast cancer patients between January 1st, 2014 and December 31st, 2017 at the level of the oncology department of the hospital public sisters Bedje of Chlef, Algeria. The incidence that has found around 43 patients per 100000 women each year. The average age of the study population is around 27 years old and the average age of patients is around 51 years old. We estimated that mammography is more used with 63, 25 % by bringing the others screening and diagnostic devices to the region of Chlef in Algeria. Studies are done worldwide since the discovery of cancer show that prevention and the different care and treatments play an important role in reducing cancer worldwide. The correct advice and guidance play an effective role in reducing this disease especially in the early stage.

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### P-193. Wide-field single photon counting detector and its applications

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Conventionally, time-domain fluorescence lifetime imaging (FLIM) is performed with a help of confocal scanning microscope and point photon counting detector. Here we present a camera system based on a position sensitive photon counter that enables time-domain FLIM acquisition with virtually any fluorescence microscope. Together with an introduction of the camera we provide a summary of several scientific applications. The first one is long-term observation of autofluorescence of living cells, which reveals dynamic changes of the internal cellular state visualized as binding and synthesis processes of NADH molecules. The second application exploits the effect of metal induced energy transfer (MIET) to measure the 3D structure of the cellular surface with nm accuracy. The third application shows imaging of a FRET biosensor inside living lymphocytes. Fourth, we present 3D-FLIM by Lightsheet microscopy. Last but not least, we demonstrate 4D hyper-spectral image acquisition where a significant reduction of fluorescence pollution of Raman signal is achieved through fluorescence decay analysis.

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### P-194. Energetic study for the induced uptake of particles into synthetic membrane systems

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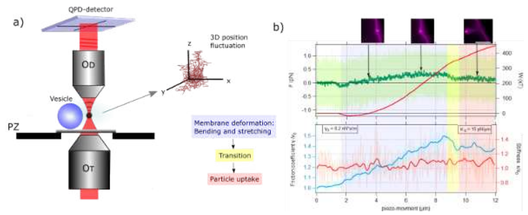
Professional phagocytes cells, like macrophages, undergo mechanical deformation in order to accomplish their defense and clearance duties. Likewise, non-phagocytic cells are prone to pathogen invasion in which they are penetrated by bacteria, viruses and fungi. All these cellular process are distinct and based on different biological uptake routes. However, at the onset of all of them, the plasma membrane is the first physical barrier to overcome to achieve a successful internalization of the external agent. The variety and vital relevance of these cellular processes demands on an interdisciplinary approach to understand the physical basis of how the external forces redefine the energetic landscape of the membrane during uptake events. In this work we use Giant Unilamellar Vesicles (GUVs) to study the forces and energies that govern the particle uptake into vesicles.

Our approach encompasses experimental studies using the Photonic Force Microscopy (PFM) technique and a mathematical model to describe the deformation energy as a function of the geometrical equilibrium shapes and mechanical properties of the vesicle. The PFM technique combines an optical tweezers setup with a back-focal-plane interferometry system, which allows three-dimensional position detection of the optically trapped particle with nanometric precision at sampling rates of Mhz as shown in figure 1a). We identified and characterized the different steps of the uptake process and correlate them with the changes in the friction and stiffness of the system, which are measured by the position fluctuations of the particle within microsecond scales, see figure 1b).

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### P-195. Kinetic and Thermodynamic Characterization of Pi-Cation Interactions for Galectin-3 by various biophysical tools

Thomas Neumann[1]

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A combination of biophysical and structural techniques allowed characterizing and uncovering the mechanisms underlying increased binding affinity of lactosamine derivatives for galectin 3. In particular, complementing information gathered from X-ray crystallography, native mass spectrometry, isothermal microcalorimetry, Biacore SPR MST and others is compared to each other.

Our studies showed favorable enthalpic contribution of cation-pi interaction between lactosamine aryl substitutions and arginine residues from the carbohydrate recognition domain, which resulted in two log increase in compound binding affinity. This incrementing strategy allowed individual contribution of galectin inhibitor moieties to be dissected. Altogether, our results suggest that core and substituents of these saccharide-based inhibitors can be optimized separately, providing valuable tools to study the role of galectins in diseases.

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### P-196. From Antibunching to Diffusion - SiMFS-Tk: A Single Molecule Fluorescence Simulator Toolkit

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To date, several dedicated simulation programs for single-molecule fluorescence experiments are available. They mostly address a specific problem domain like particle diffusion or FRET. We present SiMFS-Tk, a single molecule fluorescence simulator toolkit with a modular and extendable design. It supports simulation settings from a single energy diagram to complex multimolecular FRET experiments in solution. It also addresses multistate photophysics, including higher excited singlet and triplet states. Build upon simple command line tools, it allows fine grained control of all simulation parameters, even when running larger and more advanced jobs. SiMFS-Tk is a project targeted at all major platforms and provides a starting point for an open source repository of reusable and compatible single molecule simulation tools.

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### P-197. Does inter-loci complex formation of M.tuberculosis WXG100 proteins expand the bacteriums virulence toolbox

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Tuberculosis is a very critical infectious disease that is caused by Mycobacterium Tuberculosis, one of the most successful human pathogens. Through the many emerging antibiotic resistance strains of M. tuberculosis, so called XDR or MDR strains, particular in case of co-infection with HIV, tuberculosis is one of the major threats of the public health worldwide. The pathogen has a remarkable ability to cope with and evade the host immune system. Despite of the intensive study on tuberculosis, the underlying dynamic interplay between bacteria and host over the course of infection could not be described in molecular detail until today. Extensive research on host-pathogen interaction mechanism has identified a specialized secretion system of mycobacteria, named the Type VII (T7SS) or ESX secretion system. It is dedicated to the secretion of the well-known T-cell antigens Culture Filtrate Protein 10 kDa (CFP10 , EsxB) and Early Secreted Antigen 6 kDa (ESAT6, EsxA). The two proteins belong to the family of WXG100
proteins. It has been reported that the ESAT-6/CFP-10 complex plays an important role in the pathogenicity of the bacteria. Although the precise molecular functions of these two secrete proteins are unknown, they have to play an important role due to the fact that there are 11 EsxB- and EsxA-like gene pairs encoded in the genome of M. tuberculosis and five pairs occur within their own ESX secretion system (ESX1–5). We determined the atomic structure of this complex and a similar complex of another pathogen, S. agalactiae. WXG100 proteins share a common fouralpha helical heterodimeric structure, in spite of their low sequence conservation. Potentially, eleven native i.e cis-loci WXG100 heterodimers can be exploited by M. tuberculosis as virulence factors. Using the single molecular fluorescence method FRET, we could detect the formation of trans-loci WXG100 heterodimers. These results could be cross-validate using independent methods, chemical cross-linking and subsequent mass spectrometry analysis. We have produced five of the eleven pairs of recombinant WXG100 proteins from M. tuberculosis and are now able to explore lots of interesting experiments, including potentially developing diagnostic tools. As an outlook, we ask the speculative question, whether the eleven pairs of WXG100 could form a total of 121 combinatory heterodimers and thereby broaden the range of host immune system modulation during the course of infection.

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### P-198. Comparison of smFRET and trFRET with dsDNA as the model system

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We compared the two population-sensitive FRET methods, smFRET and trFRET, using double stranded deoxyribonucleic acid (dsDNA) with different donor-acceptor-distances and -pairs as the model system. In contrast to smFRET, for low transfer efficiencies the time resolved method was not able to distinguish between the donor only population and a large donor-acceptor-distance. The same behavior was observed for samples with different distances of the same donor acceptor-pairs, where trFRET was not able to reveal different populations. However, for distances within the Förster radius, both methods showed comparable results. Furthermore, the time resolved measurements provide indications of fast dynamics, which are not observable with the single molecule method.

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### P-199. STED for Life Sciences at Bayer

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Super-resolution microscopy (or nanoscopy) techniques enable the visualization of structures smaller than the diffraction limit of light microscopy. In the Life Science Collaboration Project (LSCP) at Bayer we are scrutinizing the applicability of STED (STimulated Emission Depletion) microscopy for different research projects in collaboration with the Pharma, Animal Health and Crop Science branches. Using STED we are focusing on the observation of subcellular localization of active ingredients and drugs, analyze the interactions between binding partners, and characterize the Mode of Action of different compounds. Furthermore, structure elucidation is carried out by means of STED microscopy. In addition, we compare STED with other nanoscopy techniques such as STORM (Stochastic Optical Reconstruction Microscopy) and SIM (Structured-Illumination Microscopy) in terms of their individual applicability and their technical possibilities and limitations.

Initial experiments employing STED microscopy have demonstrated that the structures and compounds of interest can be successfully labeled for STED applications. As a next step, we perform sophisticated data analysis as well as live-STED investigations in order to obtain a more accurate picture of a) how the compounds of interest affect subcellular processes in living cells (MoA studies) and b) the structural information. Based on these preliminary results, STED has the potential to drive decision making and to shorten product development time at Bayer.

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### P-200. How fluorescent tags modify oligomer size distributions of the Alzheimer-peptide Aβ(1-40)

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Within the complex aggregation process of Aβ-peptides into fibrils, oligomeric species, play a central role and reveal fundamental properties of the underlying mechanism of aggregation. In particular, low molecular weight aggregates have attracted increasing interest because of their role in cytotoxicity and neuronal apoptosis, typical of aggregation related diseases. One of the main techniques used to characterize such early stages of aggregation is fluorescence spectroscopy. To this end, Aβ-peptide chains are functionalized with fluorescent tags, often covalently bound to the disordered N-terminus region of the peptide, with the assumption that functionalization and presence of the fluorophore will not modify the process of self-assembly nor the final fibrillar structure. Up to date, experimental findings reveal size distributions of thermodynamically stable oligomers ranging from very narrow distributions of dimers to octamers, to very broad distributions up to 50-mers. In the present investigation we systematically study the effects of five of the most commonly used fluorophores on the aggregation of Aβ(1-40)-peptides. Time-resolved and single-molecule fluorescence spectroscopy have been chosen to monitor the oligomer populations at different fibrillation times, TEM, AFM and X-ray diffraction to investigate the structure of mature fibrils. While the structures of the mature fibrils were only slightly affected by the fluorescent tags, the sizes of the detected oligomeric species varied significantly depending on the chosen fluorophore. In particular, we relate the presence of high molecular weight oligomers (as found for the fluorophores HiLyte 647, Atto 647N and Atto 655) to net-attractive, hydrophobic fluorophore-peptide interactions, which are weak in the case of HiLyte 488, and Atto 488. The latter form low molecular weight oligomers only. Our findings reveal the potentially high impact of the properties of fluorophores on transient aggregates which needs to be included in the interpretation of experimental data of oligomers of fluorescently labeled peptides.

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## Poster Presentations

### P-201. Effect of End-Capping Motifs on the Dynamics, Stability and Mechanism of the Helix-Coil Transition.

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Previous results revealed that equilibrium helix–coil dynamics are governed by a one-dimensional diffusion of the helix-coil boundary along the peptide backbone, which was reproduced in simulations using a kinetic version of the linear Ising model. Moreover, it could be demonstrated that boundary diffusion can be described by a classical, Einstein-type, 1-D diffusion process. Recently, the effect of capping motifs on helix stability and dynamics in the center was analyzed. It was shown that capping motifs increase helix stability non- locally by altering helix unfolding but not refolding in the helix center. Up to date, the molecular origin of this non-local effect was unknown. It was suggested that capping motifs either elongate the helix, which leads to increased diffusion distances from the helix-coil boundary to the center, or slow down boundary diffusion by increased energy barriers. We investigated the effect of differently charged capping motifs located at the N- cap and C-cap position on the diffusion coefficient D and diffusion distances $\langle l^2 \rangle$ for boundary diffusion by triplet-triplet-energy-transfer (TTET). It could be demonstrated that a negatively charged N-cap (N- succinyl) and positively charged C-cap (C-2-aminoethylamide) leads to a global stabilization of the helix, whereas a positively charged N-cap (N-$NH_3^+$) leads to a global destabilization of the helix. The effect of both capping motifs on $k_u$ could be attributed to an impact on the diffusion distance $\langle l^2 \rangle$ for boundary diffusion, which was reproduced by predictions based on the Lifson-Roig theory. However, capping motifs do not affect the diffusion coefficient D. Temperature-dependent measurements confirmed that capping motifs do not alter the energy barrier encountered by boundary diffusion. Moreover, we could demonstrate that the properties of helix stabilization and destabilization can be screened by 1 M NaCl, which indicates that charged capping motifs stabilize α-helices by electrostatic interactions with the helix dipole and not by hydrogen bond interactions with unsatisfied backbone NH donor and CO acceptor groups at the helix ends. Thus, attractive electrostatic interactions between capping motifs and the helix dipole lead to an elongation of the helix, whereas repulsive electrostatic interactions lead to a decrease in helix length.

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## Poster Presentations

### P-202. Monitoring metabolic states and cell dynamics by low-light wide-field fluorescence lifetime imaging microscopy of NAD(P)H

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The fluorescence of the cofactor NAD(P)H is suitable as a non-invasive indicator for monitoring the energy metabolism of living organisms and the metabolic dynamics over time, without requiring any induction of external fluorescence labeling. Generally, cells are very sensitive to the intensity of illumination, especially in the UV range, which may change the natural behavior or even damage the cells. Here, we demonstrate a highly sensitive wide-field fluorescence lifetime imaging approach, which allows long-term observation of metabolic dynamics under extremely low-light conditions.

The metabolism of eukaryotic cells was studied by monitoring the autofluorescence of NAD(P)H. Different metabolic states and changes in the intrinsic periodic activity were identified by recording the intensity and the fluorescence lifetimes of NAD(P)H. The analysis of the fluorescence decays revealed the presence of distinctive subpopulations of NAD(P)H. The dynamics of these subpopulations will be discussed.

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