# BP 12: Single Molecule Biophysics

Time: Monday 15:00–17:15 Location: H45

**Invited Talk** BP 12.1 Mon 15:00 H45

**Imaging of G-protein coupled receptors while quantifying their ligand-binding free energy landscape to mutiple ligands** — •Daniel J. Müller[1], David Alsteens[1,2], Moritz Pfreundschuh[1], Patrizia M. Spoerri[1], Shaun R. Coughlin[3], Cheng Zhang[4], and Brian K. Kobilka[4] — [1]ETH Zurich, Switzerland — [2]University Leuven, Belgium — [3]University of California San Francisco, USA — [4]Stanford University School of Medicine, USA

Imaging native membrane receptors and testing how they interact with ligands is of fundamental interest in life sciences, but has proven remarkably difficult to accomplish. Here, we introduce experimental and theoretical developments that allow atomic force microscopy (AFM) to simultaneously image native human protease-activated receptors (PAR1) in the functionally important lipid membrane and to quantify their dynamic binding strength to native and synthetic ligands. These binding strengths provide kinetic and thermodynamic parameters of individual ligand-receptor complexes. Recorded in the absence and presence of antagonists, the values describe the ligand-binding free energy landscape of native and synthetic ligands to the G-protein-coupled receptor with remarkable accuracy. We further address the challenge and introduce multifunctional high-resolution AFM to image PAR1 and to simultaneously localize and quantify their binding to two different ligands. Our nanoscopic method opens an exciting avenue to directly image and characterize ligand-binding of native membrane receptors.

BP 12.2 Mon 15:30 H45

**How RNA Polymerase II elongates through di-nucleosomal DNA?** — Veronika Fitz[1,2], •Jaeoh Shin[3], Vasily Zaburdaev[3], and Stephan Grill[1,2,3] — [1]Max-Planck-Institute of Molecular Cell Biology and Genetics, D-01307 Dresden — [2]Technical University Dresden, BIOTEC, D-01307 Dresden — [3]Max-Planck-Institute for the Physics of Complex Systems, D-01187 Dresden

RNA polymerase II (Pol II), an enzyme which catalyzes messenger RNA from DNA template, plays a fundamental role in gene regulation. In eukaryotic cells, a large fraction of the DNA molecules are wrapped around nucleosomes, which interfere with the transcription process. Here we study Pol II elongation in di-nucleosomal template by using the optical tweezer setup. Our goal is to understand the role of the neighboring nucleosome on the Pol II elongation, which is relevant in the context of in vivo situation. We found that the Pol II elongation through the first nucleosome depends on the separation between the nucleosomes in a non-monotonous way. We suggest that this effect results from the relative angle between the nucleosomes. To better understand the experimental results, we develop a 2-dimensional random walk model accounting the nucleosomal barrier, backtracking, and the assisting force acting on Pol II. The model reproduces the dynamics of Pol II elongation in agreement with the experimental data without any fitting parameter. Out model shows that the relative strength of the nuclesomal barrier and the assisting force dramatically change the elongation dynamics. We also discuss how the second nucleosome affects the stability of the first nucleosome.

BP 12.3 Mon 15:45 H45

**Single-molecule protein nanomechanics of the chaperone DnaK** — •Gabriel Zoldak and Matthias Rief — Physik-Department E22, Technische Universität München James-Franck-Str. 1 85748 Garching Germany

In the last few years, single molecule force spectroscopy has become recognized as an excellent and unique method for probing energy landscapes of large conformational changes in proteins, including protein folding and ligand binding. Conceptually, the force spectroscopy can be applied as a tool for: (1) monitoring single-molecule kinetics with exceptional resolution, and (2) characterizing local mechanics. In the first study, we expand the dynamic range of single-molecule force spectroscopy using optical tweezers by autocorrelation analysis which pushes the time resolution of single-molecule force spectroscopy to ca. 10 microseconds thus approaching the timescales accessible for all-atom molecular dynamics simulations. In the second study, we elucidate the energetic and mechanical changes within the subdomains of the nucleotide binding domain (NBD) of the heat shock protein of 70 kDa (Hsp70) chaperone DnaK upon nucleotide binding. In an integrated approach using single molecule optical tweezer experiments, loop insertions, and steered coarse-grained molecular simulations, we find that the C-terminal helix of the NBD is the major determinant of mechanical stability, acting as glue between the two lobes. After helix unraveling, the relative stability of the two separated lobes is regulated by ATP/ADP binding.

**30 min break**

BP 12.4 Mon 16:30 H45

**Kinesin-1 motors throw each other off the microtubule** — •Matthias Rank and Erwin Frey — Arnold Sommerfeld Center for Theoretical Physics (ASC) and Center for NanoScience (CeNS), Department of Physics, Ludwig-Maximilians-Universität München

Inside cells, a large number of molecular motors bind to microtubules (MTs) where they perform directed motion. While it is generally accepted that steric hindrance of motors leads to crowding effects, little is known about their specific interactions. We examine a variation of the totally asymmetric simple exclusion process with Langmuir kinetics (TASEP/LK), in which dissociation of molecular motors is enhanced on crowded filaments. We demonstrate that a two-particle "throwing out" interaction can explain recent experimental data for kinesin-1 excellently. Following a theoretical mean-field approach, we derive the phase diagram of the process for important limits. We further extend our results to motors of two different types, and propose an efficient mechanism how they can phase separate on the MT. This can be important for cellular processes requiring ongoing catalytic activity of a specific motor type, and motors which operate cooperatively.

BP 12.5 Mon 16:45 H45

**Expanding the Design Space of Synthetic Membrane Pores** — •Kerstin Göpfrich[1], Satya Bhamidimarri[2], Alexander Ohmann[1], Iwona Mames[3], Eugen Stulz[3], Mathias Winterhalter[2], and Ulrich Keyser[1] — [1]University of Cambridge, UK — [2]Jacobs University Bremen, Germany — [3]University of Southampton, UK

DNA nanotechnology allows for the creation of membrane-spanning channels with customized functionality. We present three novel designs with channel diameters spanning an order of magnitude from 0.8nm to 8nm. We utilize DNA tile assembly and scaffolded origami with two different scaffold lengths to create channels of variable size and architectural complexity. Bifunctional porphyrin- and cholesterol-tags serve as membrane anchors to facilitate insertion into lipid membranes (J. R. Burns, K. Göpfrich et al., Angew. Chemie, 2013). We compare the conductance of the channels and confirm the correspondence between engineered design and single-channel behaviour. Our channels span three orders of magnitude in conductance, comparable to protein pores encompassing small ion channels as well as large porins. Conductance states are dependent on transmembrane voltage (A. Seifert, K. Göpfrich et al., ACS Nano, 2014). We demonstrate that self-assembly and membrane attachment of simple DNA channels can be achieved within a minute, making their creation scalable for applications in biology (K. Göpfrich et al., Nanoletters, 2015). Our work showcases the versatility of artificial DNA-based pores inspired by the rich structural and functional diversity of natural membrane components.

BP 12.6 Mon 17:00 H45

**A coarse grained DNA model for the prediction of current signals in DNA translocation experiments** — •Florian Weik — Institut für Computerphysik, Universtität Stuttgart, Germany

We present a coarse grained model of DNA in a cylindrical nanopore. It qualitatively reproduces the current modulation in a pore when DNA is present. It extends previous coarse grained and mean field approaches by incorporating a position dependent friction that was shown to be essential to the behavior by Kesselheim et. al. The experimental data of Smeets et. al. as well as the atomistic simulation results by Kesselheim et. al. are reproduced over a wide range of salt concentrations. The model reduces the computational effort by orders of magnitude as compared to all atom simulations and provides a promising starting point into a modeling of the whole translocation process. We combine a representation of the DNA by three beads per base pair with explicit ions, a heuristic friction between the DNA beads and the ions and a continuum approach of the solvent to a correct description of the electrokinetics of the system.