# BP 15: Single-Molecule Biophysics I

Time: Tuesday 10:15–13:00 Location: ZEU 250

**Invited Talk** BP 15.1 Tue 10:15 ZEU 250
**Single-molecule mechanics: theory, analysis, interpretation** — •Olga Dudko — UC San Diego, La Jolla, California, USA

Single-molecule biophysical tools permit measurements of the mechanical response of individual biomolecules to external load, revealing details that are typically lost when studied by ensemble methods. An analytical theory of single-molecule force experiments will be presented. The proposed theoretical procedure, based on a picture of diffusive crossing of a free energy barrier, provides estimates of the intrinsic rate coefficient, the location of the transition state, and the free energy of activation. A quantitative, model-free relation between the data collected in two types of measurements - under constant force and under constant force ramp speed - is established. The theoretical procedure of analyzing and interpreting experimental data will be illustrated with the unzipping of individual nucleic acid-based structures by nanopores and optical tweezers and with the unfolding of individual proteins by an atomic force microscope. Effects of multidimensionality of the free energy landscape of the biomolecule on the nature of its response to force will be explored. The theory is applicable to biological contexts ranging from protein folding to ligand-receptor interactions.

BP 15.2 Tue 10:45 ZEU 250
**A versatile first passage framework for the theoretical analysis of nanopore translocation experiments with structured polynucleotides** — •Severin Schink[1], Karen Alim[2], and Ulrich Gerland[1] — [1]ASC and CeNS, Ludwig-Maximilians-Universität Munich, Germany — [2]Harvard School of Engineering and Applied Sciences, Harvard University, USA

Probing the structures and folding dynamics of DNA or RNA molecules by translocation through nanopores is an emerging new experimental approach of single-molecule biophysics. The nanopore allows single- but not double-strands to pass and thereby couples translocation to unfolding (and refolding) of the molecule. For the quantitative interpretation of these measurements, analysis based on theoretical models for the translocation process is required. The spectrum of available theoretical approaches ranges from generic Kramers rate theory to detailed simulations of both the basepairing and translocation dynamics. Here, we present a versatile mesoscopic framework, which is based on the construction of squence-dependent one-dimensional free energy landscapes starting from the known free energy parameters for RNA and DNA secondary structure formation. This approach has only a small number of adjustable parameters which can be calibrated using translocation experiments with simple sequences. The model then yields a baseline prediction for other sequences, based on which the corresponding experiments can be interpreted, both for constant force measurements as well as nanopore force spectroscopy data. We illustrate the use of our framework with several examples.

BP 15.3 Tue 11:00 ZEU 250
**Folding quantization of a biopolymer translocating through nanopores based on multiscale simulations** — •Maria Fyta[1,2], Simone Melchionna[1,3], Massimo Bernaschi[4], Sauro Succi[4,5], and Efthimios Kaxiras[1,5] — [1]Department of Physics and School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA — [2]Physics Department, Technical University of Munich, 85748 Garching, Germany — [3]INFM-SOFT, Department of Physics, University of Rome La Sapienza, Rome, Italy — [4]Istituto Applicazioni Calcolo, CNR, Rome, Italy — [5]Initiative in Innovative Computing, Harvard University, Cambridge, MA, USA

Our recently developed novel multiscale approach which concurrently couples a mesoscopic fluid solvent with molecular motion has been efficiently applied to the problem of biopolymer translocation through narrow and wide pores. Our results of up to 10$^4$ biopolymers provide valuable insight into the cooperation of the biopolymer and hydrodynamic motion. For wide pores, capable of hosting multiple polymer strands, there is clear evidence of folding quantization, leading to a deviation from the single-exponent power-law characterizing the single-file translocation through narrow pores. The translocation proceeds through multi-folded configurations, characterized by a well-defined integer number of folds. In this case, the translocation time acquires a dependence on the average value of the folding number, leading to a deviation from the single-exponent power-law characterizing the single-file translocation through narrow pores. We discuss some recent results when electrokinetic effects are also considered.

BP 15.4 Tue 11:15 ZEU 250
**Magnetic Torque Tweezers: Probing the torsional properties of DNA, RNA, and DNA filaments** — •Jan Lipfert, Gary Skinner, Matthew Wiggin, Jacob Kerssemakers, and Nynke Dekker — Department of Bionanoscience, Delft University of Technology, The Netherlands

The double-stranded nature of DNA links cellular processes such as replication, transcription, and repair to rotational motion and torsional strains. Here we present a novel implementation of magnetic tweezers, magnetic torque tweezers (MTT), that enables the direct measurement of torque [1]. The MTT torque measurement is based on a tracking protocol that monitors x, y, z, and angle and on a redesigned magnet configuration. We have applied the MTT to DNA, RNA, and RecA-DNA heteroduplex filaments. We find the effective torsional stiffness of dsDNA to be significant force-dependent, reconciling previous partially conflicting measurements. Torque measurements on RecA-DNA heteroduplex filaments reveal an initial torsional stiffness about two-fold higher than that of DNA. However, at relatively moderate torques further build-up of torsional strain is prevented by structural transitions in the filament. Preliminary results on the torsional properties of fully double-stranded RNA indicate static properties overall similar to dsDNA, but significantly different dynamics of supercoil formation. Finally, we present a related magnetic tweezers approach that allows straight-forward measurements of free rotation, termed freely-orbiting magnetic tweezers [2]. [1] Lipfert, et al. Nature Methods (2010) [2] Lipfert, Wiggin, et al. Nature Methods, under review

**15 min. break.**

BP 15.5 Tue 11:45 ZEU 250
**Single-molecule spectroscopy on pigment proteins and bionano hybrids** — •Marc Brecht[1], Robert Bittl[2], Jana Nieder[2], and Martin Hussels[1] — [1]Universität Tübingen Institut für Physikalische und Theoretische Chemie Auf der Morgenstelle 18 72076 Tübingen — [2]FU Berlin Fachbereich Physik Arnimallee 14 14195 Berlin

I will present low temperature single-molecule fluorescence experiments on photosystem I (PSI) and PSI coupled to nano structures. The spectra show even at low temperature changes of the fluorescence emission during time like line hopping, anti-correlated intensity fluctuation or line broadening. Those changes are due to small conformational changes within the binding site of the pigments [1]. The influence of metal-nanostructures on the fluorescence properties of photosystem I, serving as an example for a multi-chromophore FRET-coupled system, will be shown [2]. Beside fluorescence-enhancement significant changes of the characteristic fluorescence emission from PSI were observed. These changes indicate altered energy transfer within the multi-chromophore assembly affecting the functionality of this protein complex. The observed spectral changes are discussed in a general framework of plasmonic interaction with multi-chromophore systems.

[1] Brecht, M. Radics, V. Nieder, J.B. and Bittl, R. (2009), PNAS, 106 (29):11857-11861 [2] Nieder, J.B. Bittl, R. Brecht, M. (2010) Angewandte Chemie,dx.doi.org/10.1002/anie.201002172

BP 15.6 Tue 12:00 ZEU 250
**Scanning evanescent fields in TIRF microscopy using a single point-like light source and a DNA worm drive** — •Hergen Brutzer, Friedrich W. Schwarz, and Ralf Seidel — Biotechnologisches Zentrum, TU Dresden, Tatzberg 47/49, 01307 Dresden

Total internal reflection fluorescence (TIRF) microscopy is an elegant technique that limits the dimension of the excitation volume along the z-direction to the hundred nanometer-scale. The method makes use of the evanescent field arising when light is totally internally reflected at the boundary to a medium of lower refractive index. Often the penetration depth of this exponentially decaying field is left undetermined limiting the reproducibility in different experiments. We directly measure this quantity by using a quantum dot as a point-like light source and a Holliday junction as a drive to move the fluorescent probe with nanometer precision along the z-direction. The junction serves as a

worm drive, which couples rotation into translational movement, while the DNA pitch serves as an intrinsic ruler. The junction is forced to migrate by adding negative turns to the DNA stretched perpendicular to the surface using magnetic tweezers. This causes the quantum dot, which is attached upstream of the junction, to decrease its height above the surface by 3.4 nm per turn. Thus it can be moved continuously through the excitation field while monitoring its height-dependent fluorescent signal. Since the quantum dot is a point-like light source, the intensity decay of the evanescent field can be obtained by dividing the signal recorded in TIRF illumination by the one recorded in conventional epi-illumination without further corrections.

BP 15.7 Tue 12:15 ZEU 250

**Weak kinesin-8 steps and slips on Microtubules** — •Anita Jannasch[1], Marko Storch[2], Jonathon Howard[2], and Erik Schäffer[1] — [1]Nanomechanics Group, Biotechnology Center, TU Dresden, Tatzberg 47-51, 01307 Dresden, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany

The budding yeast kinesin-8 motor, Kip3p, is a very processive plus-end directed motor protein. In addition, Kip3p is a microtubule length-dependent depolymerase. Here, we studied the operation of Kip3p under load using optical tweezers as a force and position sensitive tool. We expressed and purified the recombinant Kip3p-GFP fusion protein and attached it to a microsphere surface via a polyethylenglycol linker preserving its full functionality. Our studies show that single Kip3p can carry cargo with a stall force of 1.2 pN while moving with 8 nm steps along the microtubule. The velocity of the motor strongly depended on the load force. Furthermore, the motor started to slip under load. Compared to conventional kinesin, Kip3p is a slow and weak motor, which might be a trade-off for its high processivity.

BP 15.8 Tue 12:30 ZEU 250

**CXCR4-SDF1 mediated chemotaxis on the single molecule level** — •Susanne Fenz[1], Cassandra Verheul[1], Ewa Snaar-Jagalska[2], and Thomas Schmidt[1] — [1]Leiden Institute of Physics, Leiden University, The Netherlands — [2]Leiden Institute of Biology, Leiden University, The Netherlands

Directed cell movement in a chemical gradient, chemotaxis, is not only a prerequisite for many vital processes like the immune response, but also the basis for cancer spreading in metastasis. Chemotaxis is governed by extracellular gradients of small molecules, the chemokines. The receptor CXCR4 and its chemokine SDF1 play a crucial role in directing migration of tumor cells to neighbouring tissue as well as in metastasis to distant sites in the body. Two potential ordering parameters, the receptor mobility and cytoskeleton-induced membrane domains, were investigated on a molecular level in living fibroblasts and endothelial cells. We applied single-molecule fluorescence microscopy to characterize the diffusion behaviour of CXCR4-eYFP in resting cells and upon stimulation with SDF1. Particle Image Correlation Spectroscopy yields two fractions of receptors prior to stimulation: half of the receptors are immobile while the other half exhibits free diffusion with D = 0.3 mum2/s on short timescales (up to 100 ms). At longer timescales the receptors show confined diffusion within micrometer domains. Global stimulation with SDF1 switches a subset of the receptors from the immobile to the mobile fraction. We hypothesize that the impact of a gradient of SDF-1 might lead to asymmetric receptor diffusion and subsequently polarized cell behavior.

BP 15.9 Tue 12:45 ZEU 250

**Tracking single FoF1-ATP synthases in a living E. coli cell** — •Marc Renz, Torsten Rendler, Andrea Zappe, Jörg Wrachtrup, and Michael Börsch — 3.Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany

We measured the diffusion of single fluorescence-marked FoF1-ATP synthases in the plasma membrane of living E. coli bacteria. The biological questions of temporary clustering and interactions with other membrane proteins are investigated. We have built a total internal reflection fluorescence microscope for imaging single molecules in Iiving cells. The membrane protein FoF1-ATP synthase has been fluorescently labeled by two different approaches using small fusion proteins. Because of the size (2000 nm x 500 nm) and the shape of the bacterial membranes data analysis of the diffusing proteins is complicated. The algorithm used to extract the Mean Square Displacement (MSD) from the processed raw data will be discussed which allows to calculate a diffusion coefficient. The influence of the finite size of the observation area on the statistics of the measured MSD will be discussed and compared to Monte Carlo simulations.