BP 5: Single Molecule Biophysics

Time: Monday 15:00–17:30 Location: HÜL 386

Invited Talk BP 5.1 Mon 15:00 HÜL 386

Metal Induced Energy Transfer — •Jörg Enderlein — 3. Physikalisches Institut, Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen

Classical fluorescence microscopy is limited in resolution by the wavelength of light (diffraction limit) restricting lateral resolution to ca. 200 nm, and axial resolution to ca. 500 nm. However, recent years have seen a tremendous development in super-resolution techniques, such as Photoactivatable Localization Microscopy (PALM) or STochastic Optical Reconstruction Microscopy (STORM), pushing the lateral resolution down to a few nanometers. However, even with these methods, the resolution along the optical axis is typically a factor 3 to 5 worse than the lateral resolution. Recently, we have developed a new method for localizing fluorescent emitters along the optical axis with nanometer accuracy. The method is based on the energy transfer of the excited fluorophore into surface plasmons of a thin metallic film, which is extremely sensitive on the distance between the fluorophore and the metal surface. We call this method Metal Induced Energy Transfer or MIET imaging. I will explain the physical foundations of MIET, and will present numerous of its applications.

BP 5.2 Mon 15:30 HÜL 386

Dynein's direction-dependent microtubule-binding strength is controlled via a tension-induced sliding of dynein's stalk helices mediated by the coiled-coil strut — Lu Rao[1], Florian Berger[2], Matthew Nicholas[1], and •Arne Gennerich[1] — [1]Albert Einstein College of Medicine, Bronx, USA — [2]Rockefeller University, New York, USA

Cytoskeletal motor protein motility requires coordination of ATPase and filament-binding cycles. Mechanical tension strongly influences these processes, and likely regulates motor stepping as external forces resist motor movement and intramolecular tension develops between motor domains. In cytoplasmic dynein, an AAA+ ATPase, applied tension affects microtubule (MT)-binding strength anisotropically - backward tension induces stronger binding- while in the absence of tension, reconfiguration of the coiled-coil 'stalk' (which connects the AAA+ and MT-binding domains) is known to alter MT affinity. Using optical tweezers, mutagenesis, and chemical cross-linking, we show that preventing relative motion of the stalk helices or deleting the 'strut' (which emerges from the AAA+ domain and contacts the stalk) both eliminate tension-based regulation of MT-binding strength. Thus, tension alters dynein's MT-binding strength by inducing sliding of the stalk helices, and the strut is a key mediator of this process.

BP 5.3 Mon 15:45 HÜL 386

Coiled Coils as Mechanical Building Blocks — Melis Goktas, Patricia Lopez-Garcia, Ruby M. A. Sullan, and •Kerstin G. Blank — Max Planck Institute of Colloids and Interfaces

Coiled coils (CCs) are ubiquitous structural motifs found in many different proteins. They are made of α-helices that self-assemble into helical superstructures such as dimers, trimers and tetramers. CCs are important components of cytoskeletal and extracellular matrix proteins, highlighting their crucial role as mechanical building blocks. Despite their widespread appearance, the general structural determinants that define their molecular mechanical properties in different pulling geometries ('shear' vs. 'unzip') are largely unknown. With the goal of shedding light on the structure-to-mechanics relationship, we are applying AFM-based single molecule force spectroscopy to a set of CCs characterized by differences in CC length and sequence. We show that a 28-amino-acid-long, heterodimeric CC ruptures at a most probable force of >40 pN when loaded in shear geometry, while the rupture force for the unzip geometry is below the detection limit of AFM. In the shear geometry, we observe a clear dependence on CC length and helix propensity, showing that the rupture force of CCs in the shear geometry can be tuned by a number of parameters. Our final goal is to develop a library of CCs for the synthesis of CC-based materials with tunable mechanical properties for applications in tissue engineering.

BP 5.4 Mon 16:00 HÜL 386

3D Light microscopy of protein structure with Angstrom resolution — •Daniel Böning[1], Siegfried Weisenburger[1], Benjamin Schomburg[2], Karin Giller[2], Stefan Becker[2], Christian Griesinger[2], and Vahid Sandoghdar[1] — [1]Max Planck Institute for the Science of Light, 91058 Erlangen, Germany — [2]Max Planck Institute for Biophysical Chemistry, 37077 Goettingen, Germany

Insight into the atomic and molecular structure of proteins and other biomolecular assemblies is highly desirable in many areas of life sciences, and several physical techniques such as x-ray crystallography, electron microscopy (EM) and magnetic resonance spectroscopy have been employed over decades to arrive at such information. However, due to limitations in each method the structures of the great majority of the proteins and larger biomolecules remain unknown to us. Here, we present a novel optical microscopy technique, termed Cryogenic Optical Localization in three Dimensions (COLD), which reaches Angstrom resolution in deciphering the positions of several fluorescent sites within a single small protein. The key mechanism for reaching this limit is the enhanced photostability at low temperatures, thus providing a much higher shot-noise-limited signal-to-noise ratio than in room temperature super-resolution microscopy. As an example of the application of this method, we show how we resolve the four sites where biotin binds to streptavidin in three dimensions. COLD opens new doors for obtaining quantitative structure information from small to medium sized biomolecules at the Angstrom scale and can complement other existing techniques such as magnetic resonance spectroscopy.

BP 5.5 Mon 16:15 HÜL 386

Biased side-stepping enables single molecules of yeast kinesin-8 to avoid roadblocks on microtubules — Aniruddha Mitra[1,2], •Felix Ruhnow[1], and Stefan Diez[1,2] — [1]B CUBE - Center for Molecular Bioengineering, TU Dresden, Germany — [2]Center for Advancing Electronics Dresden (cfaed), TU Dresden, Germany

During mitosis, kinesin-8 motors regulate spindle length based on their depolymerization activity at microtubule plus-ends. Remarkably, depolymerization occurs in a length-dependent manner, the underlying mechanism of which has been described by an antenna model where motors bind along the entire lengths of the microtubules and subsequently walk to the plus-ends relying on their extremely high processivity. During such long runs, motors in vivo are expected to frequently encounter roadblocks, such as microtubule-associated proteins. It has therefore been speculated that kinesin-8 motors may use protofilament switching on the microtubules lattice to bypass such obstacles. Whereas recent reports agree that kinesin-8, quite in contrast to kinesin-1, is indeed capable of switching protofilaments, it has not been clear if the underlying side-stepping occurs in a directionally-biased manner. To resolve this controversy, we tracked the 3D-motion of single QD-conjugated kinesin-8 motors stepping along freely suspended microtubules. We observed a spiraling motion with leftward pitches in the order of 1um, indicating that the motors do switch protofilaments in a biased manner. Assays under limiting ATP conditions and in the presence of roadblocks reveal that side-stepping is a robust phenomena, which is not directly coupled to the forward stepping rate.

BP 5.6 Mon 16:30 HÜL 386

Controling the translocation of polymers through by selective nanopore modifications — •Adwait Datar[1], Umberto Marini Bettolo Marconi[2], Simone Melchionna[3], and Maria Fyta[1] — [1]Institute for Computational Physics, University of Stuttgart, Germany — [2]School of Sciences and Technologies, University of Camerino, 62032 Camerino Italy — [3]ISC - CNR, Institute for Complex Systems, Consiglio Nazionale delle Ricerche, Università La Sapienza, P.le A. Moro 2, 00185 Rome, Italy

The focus of this work is the process of charged polymer translocation through nanometer-sized nanopores. These nanopores are placed in a salt solution and can electrophoretically thread charged molecules. Our aim is to control the polymer dynamics throughout the translocation process and optimize the translocation speed. In order to achieve this, we attempt to tune the specific polymer-nanopore interactions by changing the pore characteristics. Specifically, we investigate the influence of a variety of different patterns of the charge distribution within the nanopore on guiding the polymer dynamics. Our work is based on a multiscale computational approach seemlessly coupling the dynamics of the polymer with an electrokinetic description for the salt solution in which the translocation process takes place. Our results are evaluated with respect to different sensors characteristics in the nanopore,

the flow patterns within the pore, and the velocity of the translocated polymer, as well as the cooperativity of solution, flow, and nanopore. We discuss the impact of our work in selectively engineering nanopores for single molecule experiments and DNA sequencing.

BP 5.7 Mon 16:45 HÜL 386

Single-Molecule Biophysics: The Challenge of Reproducibility — •Fabian Czerwinski[1], Lene Oddershede[2], and Oliver Otto[1] — [1]Universität of Greifswald, Greifswald, Germany — [2]Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark

Resolving dynamic processes within inherently fluctuating systems often sets the biophysical agenda to employ novel analysis methods as well as to develop cutting-edge technology. Thus, single-molecule experiments and their vastly evolving data are faced with the scientific demands of consistency and reproducibility. This requirement is reinforced as single-molecule biophysics assesses more and more tasks of quantitative biology.

Here, we discuss three measures that allow for consistent comparison of single-molecule data: i) Bayesian likelihood, ii) Allan variance, and iii) editorial standardization. In a retrospective way, we review examples of force spectroscopy and super-resolution fluorescence microscopy. The examples are confronted with Nature's editorial checklist for life sciences articles [1, 2]. We argue that the further growing interest in biophysical single-molecule data mandates rigorous, transparent and comprehensive effort from scientists and institutions.

[1] Reducing our irreproducibility. Nature 2013, 496:398
[2] http://www.nature.com/authors/policies/checklist.pdf

BP 5.8 Mon 17:00 HÜL 386

Analytical catch-slip bond model for arbitrary forces and loading rates — •Jakob Tómas Bullerjahn and Klaus Kroy — Universität Leipzig, Institut für Theoretische Physik, Leipzig, Germany

Some biological bonds exhibit a so-called catch regime, where the bond strengthens with increasing load. We build upon recent advances in slip-bond kinetics [1] to develop an analytically tractable, microscopic catch-slip bond model [2]. To facilitate the analysis of force-spectroscopy data, we calculate the bond's mean lifetime and the rupture-force distribution for static loading and linear force ramps. Our results are applicable for arbitrary forces and loading rates, covering the whole range of conditions found in experiments and all-atom simulations. A generalization to account for force transducers of finite stiffness is also provided.

[1] J. T. Bullerjahn, S. Sturm & K. Kroy, *Nat. Comm.* **5**, 4463 (2014).
[2] J. T. Bullerjahn & K. Kroy, *PRE* **93**, 012404 (2016).

BP 5.9 Mon 17:15 HÜL 386

In vitro study of the regulation and the mechanism of the MKLp2 mitotic kinesin — •Amna Abdalla Mohammed Khalid[1], I-Mei Yu[2], Christoph Schmidt[1], and Anne Houdusse[2] — [1]Third Institute of Physics - Biophysics, Georg August University, Germany — [2]Institut Curie Paris, France

The mitotic kinesin-like protein 2 (MKLp2), is a N-terminal kinesin of the Kinesin-6 family with unique features. It plays critical roles for the metaphase to anaphase transition and for cytokinesis in cell division. Previous studies have shown that MKlp2 inhibitors reduced the cell growth in pancreatic adenocarcinoma cells and can kill tumour stem cells. On the other hand, MKlp2 is overexpressed in variety of cancers. Therefore it is considered as a good candidate for new cancer therapies. High-resolution single-molecule studies are fundamental to understand the regulation and mechanism of MKlp2 at the molecular level. We are studying truncated MKLp2 constructs to explore basic motor functions and to understand the influence the domains on motor properties. Here I present single-molecule studies of a dimeric truncated MKlp2, in vitro using total internal reflection fluorescence microscopy (TIRFM). Our data confirm that the dimeric truncated MKlp2 motors are active. A conspicuous feature of this motor is its microtubule bundling activity.