BP 10: Posters - Single Molecule Biophysics

Time: Monday 17:30–19:30 Location: P3

BP 10.1 Mon 17:30 P3

Single molecule detection in microflow — •Eleonora Perego, Viktor Schroeder, and Sarah Köster — Institute for X-Ray Physics, Georg-August-Universität Göttingen, Germany

In recent years single molecule fluorescence methods have emerged as powerful method to study assembly, aggregation and diffusion of biomolecules. We use techniques like fluorescence correlation spectroscopy (FCS) or photon counting histogram (PCH) to study these process with high spatial resolution. However these techniques are inherently "slow". Thus in order to overcome this limitation, we combine these methods with microfluidics. This allow us to study the assembly and the diffusion of biomolecules in a time-resolves manner. One application is the study of early time points of protein assembly, like intermediate filaments, that are usually difficult to measure with more classical bulk experiments. Moreover, by employing our setup, it is possible to identify the passage of a single molecule through the excitation volume by tuning the concentration and the flow conditions of the sample stream. Our results show that the combination of microfluidic and single molecule fluorescence methods provides a very suitable approach for studying the aggregation of biomolecules in real time, which is important for understanding cellular behavior.

BP 10.2 Mon 17:30 P3

Plasmonics and Nanofluidics for DNA-Single Molecule Detection — •Parisa Bayat, Irene Fernandez-Cuesta, Franziska Esmek, Thomas Kling, and Robert H. Blick — Center for Hybrid Nanostructures & Institute of Nanostructure- and Solid State Physics, University of Hamburg, Germany

Plasmonic antenna nano-focus the light beyond diffraction. These hot spots are ultra-sensitive, what can be exploited for single molecule (bio) sensing. But there is a major challenge: placing the target element at the sensitive area. Here, we have integrated a sub-100nm nanochannel crossing the antenna gap, what allows the in-line detection of single molecules of DNA in real-time as they pass through the light "hotspot". In this configuration, the molecules are detected as peaks in the fluorescent signal in time scans. This allows real time read-out of the molecules with no limitation in the length and without an expensive camera. For total liquid control, the nanochannel is connected to a complete fluidic system. This represents a new type of super-sensitive (bio) sensor, with single- molecule real time detection capabilities. We have developed a wafer-scale fabrication process, based on nanoimprint lithography [1], to make the complete fluidic devices in one single step, only 120 seconds. Discrete DNA molecules have been detected and counted by in-line detection in real time. Different types of viral DNA molecules (λ-Bacteriophage and Kaposi's sarcoma herpesvirus) were stained with intercalating dyes and stretched in the nanochannels.

[1] I. Fernandez-Cuesta et al., J. Vac. Sci. Technol. B29, 06F801 (2011)

BP 10.3 Mon 17:30 P3

Label-free iSCAT detection of secretory proteins from single living cells — •Andre Gemeinhardt[1], Matthew McDonald[1], Katharina König[1], Stefanie Schaller[2], Michael Aigner[2], Andreas Mackensen[2], and Vahid Sandoghdar[1] — [1]Max Planck Institute for the Science of Light, Erlangen, Germany — [2]Department of Haematology and Oncology, Universitätsklinikum Erlangen, Germany

Interaction and communication between cells is partly guided via an exchange of different proteins. Here, we report the detection of single unlabeled proteins secreted by individual living cells. This is realized via an interferometric scattering technique (iSCAT) developed in our laboratory that circumvents the need to fluorescently label the biomolecules under study. The technique's use of interference between a small scattering signal and a larger reference wave paves the way to detect even the smallest particles. We apply this method to analyze the secretomics of single cytotoxic T-cells under external stimulation. By varying the system between body temperature and fever-like conditions, we observe a clear trend towards shorter reaction times after triggering cytokine secretion. The developed method is a promising way to elucidate the complicated nature of immune response through the basic principles of membrane-mediated protein release and cellular exchange.

BP 10.4 Mon 17:30 P3

Mechanical Properties of Leishmania Myosin XXI determined with an Optical Tweezers based Force Transducer — •Andreas Graw, Christopher Batters, and Claudia Veigel — Department of Cellular Physiology (LMU) and Center for Nanoscience (CeNS), München, Germany

Myosins form a large family of actin-based motor proteins that are involved in different forms of cellular motility. Force and movement are generated by changes in conformation of the actomyosin complex. Myosin XXI is the only myosin shown to be expressed in Leishmania parasites and is involved in a variety of motile functions. Previous studies indicated that members of the calmodulin family regulate dimerization, motility, and lipid binding of this molecular motor.

Here we present the first mechanical measurements to determine the stiffness and working stroke of a single myosin XXI motor molecule using optical tweezers. These measurements are performed using the "three bead" geometry in which an actin filament is stretched out via two optically trapped handle beads attached to either end of the filament to form a dumbbell. The dumbbell is positioned in the vicinity of a third bead carrying the myosin motor[2]. The movements and forces produced by the actomyosin interactions are observed by detecting the position of both trapped beads with four-quadrant-photodiodes. In order to characterize the effect of the C-terminal tail on the mechanical performance of the motor we immobilized the motor using different attachment modes including physiological phospholipids.

BP 10.5 Mon 17:30 P3

Universal bound on the efficiency of molecular motors — •Patrick Pietzonka[1], Andre C. Barato[2], and Udo Seifert[1] — [1]II. Institut für theoretische Physik, Universität Stuttgart — [2]Max Planck Institute for the Physics of Complex Systems, Dresden

The thermodynamic uncertainty relation provides an inequality relating any mean current, the associated dispersion and the entropy production rate for arbitrary non-equilibrium steady states. Applying it here to a general model of a molecular motor running against an external force or torque, we show that the thermodynamic efficiency of such motors is universally bounded by an expression involving only experimentally accessible quantities [1]. For motors pulling cargo through a viscous fluid, a universal bound for the corresponding Stokes efficiency follows as a variant. A similar result holds if mechanical force is used to synthesize molecules of high chemical potential. Crucially, no knowledge of the detailed underlying mechano-chemical mechanism is required for applying these bounds.

[1] P. Pietzonka, A. C. Barato, U. Seifert, J. Stat. Mech. *in press*, arXiv:1609.08046

BP 10.6 Mon 17:30 P3

Catch bond interaction between cell surface sulfatase Sulf2 and glycosaminoglycans — Ahmet Erol[1], •Sören Grannemann[1], Christian Bartz[2], Volker Walhorn[1], Thomas Dierks[2], and Dario Anselmetti[1] — [1]Experimental Biophysics & Applied Nanoscience, Bielefeld University, Germany — [2]Biochemistry I, Bielefeld University, Germany

In biological adhesion, the biophysical mechanism of specific noncovalent biomolecular interaction can be divided in slip- and catch-bonds, respectively. Conceptually, slip bonds exhibit a reduced bond lifetime under increased external force and catch-bonds, in contrast, an increased lifetime for a certain force interval. Catch bonds therefore act in a counter intuitive manner.

Upon investigating the specific interaction between the unique hydrophilic domain (HD) of the human cell-surface sulfatase Sulf2 against its native glycosaminoglycan (GAG) target substrate heparan sulfate (HS) by single-molecule force spectroscopy (SMFS), we found clear evidence of catch-bond behavior which, by means of control experiments, could be specifically related to the GAG 6-O-sulfation site. Alongside to our previous work on the catch bond behavior of HDSulf1 [1], we analyzed the data within the theoretical framework of a force mediated transition between two coupled slip bond regimes.

[1] A. Harder et al., Biophysical Journal 108(7), p1709-1717 (2015)

BP 10.7 Mon 17:30 P3

Investigation of cardiomyopathy-related desmoglein-2 vari-

ants — •Mareike Dieding[1], Raimund Kerkhoff[1], Jana Debus[2], Anna Gärtner-Rommel[2], Volker Walhorn[1], Hendrik Milting[2], and Dario Anselmetti[1] — [1]Experimental Biophysics & Applied Nanoscience, Bielefeld University, Germany — [2]Erich und Hanna Klessmann-Institut, Herz- und Diabeteszentrum Bad Oeynhausen, Germany

Desmoglein-2 (DSG2) is a desmosomal transmembrane glycoprotein in heart muscle cells. The homophilic interaction of its extracellular domains provide the intracellular mechanical contract between the desmosomes of adjacent cells. Variants of DSG2 are associated with arrhythmogenic right ventricular cardiomyopathy (ARVC) a rare but severe heart muscle disease. DSG2 wildtype (WT) and several variants were investigated on the cellular and the single molecule level, respectively. The adhesion of single DSG2 homo-complexes was analyzed by means of atomic force microscopy based single-molecule force spectroscopy (AFM-SMFS). Moreover, using Jarzynski's equation we estimated the difference of free energy in order to fully characterize the kinetics and thermodynamics of the homophilic DSG2 binding. Furthermore, cell-cell adhesion was analyzed using confluent monolayers of stable transfected full-length-DSG2 WT and DSG2 variant HT1080 cultures that were subjected to mechanical stress.

The results of the dissociation assay and AFM experiments consistently revealed that DSG2 variants tend to an increased cell-cell adhesion and a prolonged DSG2 bond life-time, respectively.

BP 10.8 Mon 17:30 P3

Biophysical investigation of the association of histones with double- and single-stranded DNA — Ying Wang[1], •Dennis Kreft[1], Luis van Merwyk[1], Katja Tönsing[1], Volker Walhorn[1], Xavier Fernàndez-Busquets[2], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanoscience, Faculty of Physics, Bielefeld University, Bielefeld, Germany — [2]Institute for Bioengineering of Catalonia and Barcelona Institute for Global Health, Barcelona, Spain

Nucleosome formation is the process of how nucleic dsDNA is packed in eukaryotes. Nucleosome core particles consist of 147 bp of dsDNA, which wrapped in 1.67 left-handed superhelical turns around a histone octamer, consisting of 2 copies each of the core histones (H2A, H2B, H3, H4) and stabilized by the linker histone H1, forming the well-known "beads on a string" chromatin structure. Despite many studies of histones associating with dsDNA, little is known about the structures generated by the interaction of histones with ssDNA. In this work, we employed magnetic tweezers (MT) and atomic force microscopy (AFM) to investigate the association of histones with dsDNA and ssDNA. For ssDNA in the presence of histones, the results of MT assays indicate a shortening of ssDNA upon its interaction with histones as well as AFM imaging exhibits a compacted ssDNA structure. Compared with the characteristics of histones-dsDNA binding, these data suggest that histones and ssDNA associate into some type of nucleosome-like assembly that may facilitate the participation of histones in the replication and transcription of chromatin.

BP 10.9 Mon 17:30 P3

Recombinant mammalian kinesin-3, KIF16B, is not autoinhibited and moderately processive by itself — •Rahul Grover[1,2], Raluca Groza[1], Tim Rehfeldt[1], and Stefan Diez[1,2] — [1]B CUBE & cfaed, TU Dresden, Germany — [2]MPI-CBG, Dresden, Germany

KIF16B, a kinesin-3 family motor, is involved in the transport and localization of early endosomes. It exhibits a lipid-binding PX-domain, through which it directly binds to PI(3)P-containing vesicles. Recent in-vivo studies on KIF16B have proposed contradictory mechanisms for its activity and transport properties. One study proposed that KIF16B is a monomer and dimerizes only when bound to a membranous cargo, upon which it becomes activated and super-processive (run length $>$ 10 um). In contrast, another study proposed that KIF16B is autoinhibhited by its stalk domain in an ATP-dependent manner without any influence of the presence of membranous cargo or PX-domains. Thus, studying single KIF16B motors in the complex environment of a cell appears to be difficult, resulting in inconsistent postulations about its functional principles. To understand the molecular mechanism of KIF16B we carried out in-vitro assays with purified components. We expressed full-length KIF16B motors labeled with GFP and performed stepping motility assays as well as photobleaching analysis. We found that single molecules of KIF16B are active, dimeric and moderately processive (run length $<$ 2 um). Our results suggest that other auxillary proteins (e.g. Rab-family proteins) can be involved in regulating the activity of KIF16B in cells.

BP 10.10 Mon 17:30 P3

Ribonulceic acid induced fluorescence enhancement (RIFE) - Carbocynanines in the realm of RNA — Fabio Steffen, Roland K.O. Sigel, and •Richard Börner — Department of Chemistry, University Zürich, Zürich, Switzerland

The popularity of carbocyanine dyes in single molecule spectroscopy of nucleic acids is unbroken (1,2). Studying the dynamics of large RNA constructs such as the group II intron in S. Cerevisiae on the single molecule level by means of FRET (3) have motivated a thorough photophysical characterization of the FRET pair Cy3/Cy5 in context of nucleic acids and RNA in particular (4). We observe that, apart from strand composition, structural features of the biomolecule play a fundamental role in RNA-dye recognition. In this respect, secondary structure motifs and its intrinsic flexibility sets RNA well apart from DNA and justifies the introduction of RNA-induced fluorescence enhancement (RIFE) (4) as a phenomenon akin to proteins (PIFE) (5) to describe a series of photophysical effects caused by structural motifs of RNA in vicinity of an isomerizable cyanine fluorophore. Besides quantitative studies of RIFE, we outline possible applications of ALEX-based RIFE-FRET studies with immobilized RNA molecules.

(1) Börner R, Kowerko D, Guisett-Miserach H, Schaffer MF, Sigel RKO. (2016) Coord .Chem. Rev. 327-328:123. (2) Levitus M, Ranjit S. (2011) Q. Rev. Biophys. 44:123. (3) Fiorini E, Börner R, Sigel RKO. (2015) Chimia. 69(4):207. (4) Steffen F, Sigel RKO, Börner R. (2016) Phys. Chem. Chem. Phys. 18(42):29045. (5) Lerner E, Ploetz E, Hohlbein J, Cordes T, Weiss S. (2016) J. Phys. Chem. B. 120(26):6401.