BP 9: Single Molecule Biophysics

Time: Monday 16:45–18:30 Location: BAR/0205

Invited Talk BP 9.1 Mon 16:45 BAR/0205

Breaking the photobleaching limit in single-molecule FRET with nanophotonic DyeCycling. — Benjamin Vermeer[1], Dong Hoon Shin[2,3], Alexander Vogel[4], Fabian Zundel[1], Sabina Caneva[2], and •Sonja Schmid[1,4] — [1]University of Basel, Basel, Switzerland — [2]Delft University of Technology, Delft, The Netherlands. — [3]Korea University, Sejong, Republic of Korea — [4]Swiss Nanoscience Institute, Basel, Switzerland

Paradoxically, single-molecule FRET studies rely on ensemble averaging during data analysis, because early photo-bleaching prohibits sufficient sampling of single molecules. As a result, the FRET-based study of inter- and intra-molecular heterogeneity in biomolecular function - a specific hallmark of single-molecule techniques - is hardly possible, preventing insights into dynamic disorder, the effects of post-translational and other modifications, of rare but decisive states, etc. Here, we demonstrate hour-long single-molecule FRET observations using DyeCycling in zero-mode waveguides, which circumvents photobleaching through reversible fluorophore binding. We detect the conformational dynamics of single molecules over four orders of magnitude in time (milliseconds-hour), enabling us to directly observe slow kinetic regime changes within individual molecules that were intractable previously. Moreover, we demonstrate the versatility of DyeCycling with DNA and protein molecules. Together, these advances establish DyeCycling/FRET as a powerful new approach that vastly expands the information gain of single-molecule FRET, enabling the study of important biological questions that were previously inaccessible.

BP 9.2 Mon 17:15 BAR/0205

Monitoring the ribosome dynamics at the single molecule level — •Baptiste Bouhet[1], Sandra Blanchet[2], Charles Truong[3], Olivier Namy[2], and Karen Perronet[1] — [1]Light, Matter and Interactions lab, Gif/Yvette, France — [2]Institute for Integrative Biology of the Cell, Gif/Yvette, France — [3]Centre Borelli, Gif/Yvette, France

Protein synthesis is a complex multi-step process involving factors that need to interact in a coordinated manner to properly translate mRNA. As translating ribosomes cannot be synchronized over many elongation cycles, single molecule studies, mainly using total-internal-reflexion fluorescence microscopy, have been introduced to better understand translation dynamics. We decided to monitor the passage of individual, unmodified eukaryotic ribosomes from wheat germ extracts at specific fluorescent primers hybridized along mRNA. Because of the ribosome helicase activity, the double strand formed by the oligonucleotide and the mRNA is opened while the ribosome translates this region of the mRNA. Thus, the consecutive loss of fluorescence signal of two oligonucleotides allows us to measure the translation speed distribution of single ribosomes. We use this system to measure simultaneously the initiation and the elongation kinetics for linear mRNA and during -1 frameshifting, which is induced by a secondary structure on mRNA. We are also currently developing a magnetic tweezers assay to get complementary information on the opening dynamics of these structures. Thanks to its versatility, this method is a valuable tool to investigate translation machinery modifications in human diseases.

BP 9.3 Mon 17:30 BAR/0205

Single-molecule force and torque spectroscopy reveals conformational transitions in DNA and proteins — •Jan Lipfert — Universität Augsburg

Magnetic tweezers are a powerful tool to probe single molecules under precisely controlled forces, down to well below 1 pN, and, in addition can control twist and torque. Here, I highlight recent developments and applications of magnetic tweezers. In particular, I will present how we use magnetic tweezers to probe regulatory conformational changes in protein complexes, including pathogen-cell adhesion and the initiation of primary hemostasis by von Willebrand factor. In addition, I will show how we use the capabilities of magnetic tweezers to precisely measure DNA helicity under changes in environmental conditions and upon small-molecule binding.

BP 9.4 Mon 17:45 BAR/0205

Direct measurement of ultra-weak plant kinesin steps using silicon nanospheres as optical tweezers probes — •Aleksandr Kostarev[1], Shu Yao Leong[1], Anita Jannasch[1], Minoru Fujii[2], Hiroshi Sugimoto[2], and Erik Schäffer[1] — [1]Eberhard Karls Universität Tübingen, Tübingen, Germany — [2]Kobe University, Kobe, Japan

Kinesin motor proteins are essential for transport along cytoskeletal microtubules and cell division. In plants, cytokinesis relies on phragmoplast orienting kinesins (POKs). The kinesin-12 paralog POK2 can generate only about 300 fN of force. This low force is consistent with the idea that POK2 does not transport cargo, similar to weak kinesin-8 motors, the function of which is unknown in plants. To understand the function and mechanochemistry of these ultra-weak motors, it is necessary to detect their individual steps during ATP hydrolysis. However, the spatiotemporal resolution achieved with conventional probes in optical tweezers is insufficient for detecting fast steps at such low forces. To overcome this limitation, we used high refractive-index silicon nanospheres for optical trapping. With these nanospheres, we achieved an unprecedented force resolution of 60 aN at room temperature in liquids with a force sensitivity of 2.7 fN Hz^(-1/2). We bound single POK2 or plant kinesin-8 motors to the nanospheres and were able to detect individual 8-nm steps with 4-nm substeps within a force range of 100-400 fN. These in vitro measurements establish a path toward identifying plant kinesin function and demonstrate the potential of silicon nanospheres for studying ultra-weak molecular machines.

BP 9.5 Mon 18:00 BAR/0205

Ultrafast sensing of single nanoparticles with an optofluidic microcavity — •Shalom Palkhivala[1], Larissa Kohler[1], Christian Ritschel[1], Christoph Pauer[2], Tim Liedl[2], Claus Feldmann[1], and David Hunger[1] — [1]Karlsruhe Institute of Technology, Karlsruhe — [2]Ludwig Maximilian University, Munich

The characterisation of single, unlabelled particles in water is of much interest in biophysics and chemistry, where most processes occur in an aqueous environment. We report measurements of single nanoparticles in aqueous suspension using a fibre-based Fabry-Perot microcavity. For quantitative analysis of the nanoparticles' diffusion dynamics, we developed an analytical autocorrelation function to model diffusion in a standing wave field. This enabled the accurate sizing of nanoparticles having diameters down to 3 nm. [1]

Additionally, the rotational dynamics of anisotropic particles were investigated. Via the polarization modes of the cavity, the orientation of a nanorod was tracked with high temporal resolution ($\sim$ 20 ns), orders of magnitude faster than most other current techniques. As an application of our sensor to biosensing, we demonstrate measurements of individual DNA "origami" structures and of few protein molecules, which already enabled their hydrodynamic sizes to be determined.

We further expect our nanosensor to give an insight into the structural properties and conformation of single bioparticles, and to become a powerful tool for diagnosis in biomedicine and for biochemical and environmental assays.

[1] Palkhivala *et al.* (2025), *ACS Nano*, 19, 45, 39320-39326

BP 9.6 Mon 18:15 BAR/0205

Indications for Ultrafast Energy Transfer and Subsequent Disulfide Bond Cleavage in Lysozyme upon Ultrafast Excitation of Aromatic Residues — •Philip Wehling[1], Jessica Harich[1], Antonia Freibert[1], Ru-Pan Wang[2], Tae Gyun Woo[3], Junho Lee[3], Seonghyeon Jeong[3], Sungin Yu[3], Haneol Oh[3], Migel Ochmann[1], Victoria Kabanova[4], Emma Beale[4], Philip Johnson[4], Claudio Cirelli[4], Camila Bacellar[4], Briony Yorke[5], Tae Kyu Kim[3], and Nils Huse[1] — [1]University of Hamburg, Hamburg, Germany — [2]DESY, Hamburg, Germany — [3]KAIST, Daejeon, Republik of Korea — [4]Paul Scherrer Institute, Villigen, Switzerland — [5]University of Leeds, Leeds, U.K.

Disulfide bonds play a crucial role in stabilizing the tertiary structure of proteins. Ultraviolet (UV) radiation leads to S-S bond cleavage, possibly compromising the functionality of proteins. Femtosecond X-ray absorption spectroscopy at the sulfur K-edge revealed ultrafast geminate disulfide bond reformation in aliphatic disulfides [1]. These findings raise the question how disulfide bridges behave in proteins upon UV irradiation. Disulfide photochemsitry in Lysozyme by UV excitation across the dominant absorption of the aromatic residues leads to efficient sulfur radical formation which must stem from en-

ergy transfer. We discuss yields and possible mechanisms of energy transfer in competition with direct photocleavage of disulfide bridges, and implications for energy dissipation and structural integrity of proteins.

[1] M. Ochmann et al, Nat. Commun. 15, 8838 (2024).