BP 27: Single Molecule Biophysics

Time: Thursday 9:30–13:00 Location: H 0112

BP 27.1 Thu 9:30 H 0112

Metal-Induced Energy Transfer (MIET) for Live-Cell Imaging with Fluorescent Proteins — •Lara Hauke, Sebastian Isbaner, Arindam Gosh, Alexey I. Chizhik, Ingo Gregor, Florian Rehfeldt, and Jörg Enderlein — Third Institute of Physics, Biophysics, Georg August University Göttingen, Germany

Metal-Induced Energy Transfer (MIET) imaging has emerged as a versatile super-resolution technique, offering nanometer resolution along the optical axis in microscopy. While MIET has demonstrated its efficacy in various biological and biophysical studies, its potential for live-cell imaging using fluorescent proteins has yet to be fully realized. In this study, we explore the implementation of MIET imaging for live-cell studies across diverse cell types, including adult human stem cells, human osteo-sarcoma cells, and Dictyostelium discoideum cells. Our investigation encompasses a range of commonly used fluorescent proteins, such as GFP, mScarlet, RFP, and YPet. Our findings showcase the applicability and capabilities of MIET imaging in achieving nanometer axial mapping of living cellular and sub-cellular components. Importantly, we demonstrate MIET's ability to operate across multiple timescales, spanning from milliseconds to hours, while inducing negligible phototoxic effects. This work [10.1021/acsnano.2c12372] establishes MIET as a powerful and easy-to-implement tool for live-cell imaging, providing researchers with a valuable resource for non-invasive, high-resolution visualization of dynamic cellular processes in various biological contexts.

BP 27.2 Thu 9:45 H 0112

Imaging Mucin with Low Energy Electron Holography — •Moritz Edte[1,2], Ben Yang[1], Luigi Malavolti[1], and Klaus Kern[1,2] — [1]Max-Planck-Institute for Solid State Research, Stuttgart, Germany — [2]École polytechnique fédérale de Lausanne, Switzerland

The glycosylated protein family of transmembrane mucins plays an important role in living cells [1,2]. Mucin molecules show different degrees of glycosylation in healthy and cancerous cells [1,2] associated with a possible structural change. Due to the high complexity and flexibility of these molecules, which challenges state-of-the-art methods, a single-molecule imaging technique is required to study how the degree of glycosylation affects the mucin structure. In our in-house custom-built low-energy electron holography (LEEH) setup, a low-energy electron beam in the 50-150 eV energy range allows high-contrast imaging of single biomolecules deposited by electrospray ion beam deposition (ES-IBD) [3,4]. Our method allows the mapping of conformational variability of single flexible molecules [3,4]. Here, I present LEEH imaging of single mucin molecules with varying degrees of glycosylation, and show that LEEH combined with ES-IBD is able to image these flexible molecules. This study demonstrates that LEEH can be used as a complementary method to study structural features associated with conformational changes in individual biomolecules. [1]*D.W. Kufe et al., Nature Review Cancer 9, 874-885 (2009) [2]*G.C. Hansson, Annual Review of Biochemistry 89, 769-793 (2020) [3]*H. Ochner et al., PNAS 118 (51), e2112651118 (2021) [4]*H. Ochner et al., scientific reports 13, 10241 (2023)

BP 27.3 Thu 10:00 H 0112

Label-free imaging and 3D single particle tracking in complex media via interferometric scattering microscopy (iSCAT) — •Kiarash Kasaian[1,2], Mahdi Mazaheri[1,2], and Vahid Sandoghdar[1,2] — [1]Max Planck Institute for the Science of Light, Erlangen, Germany — [2]FAU Erlangen-Nürnberg, Erlangen, Germany

Label-free imaging offers a distinct advantage over techniques employing fluorescent labels by eliminating concerns related to photobleaching and photo-toxicity. iSCAT is a highly sensitive tool for label-free microscopy [1]. However, imaging complex biological specimens with iSCAT remains challenging, as the scattering from multiple sources generates dynamic speckle patterns, which can obscure the signal of interest. Introducing a novel iSCAT modality termed "diffused-illumination iSCAT" (DI-iSCAT) [2], we engineer the spatial coherence of the illumination to mitigate the impact of dynamic speckle. We demonstrate high-speed imaging of live cells over a large field of view of 100 μm × 100 μm and employ our newly developed 3D tracking algorithm [3] to perform 3D tracking of nanoparticles in cellular environment.

[1] Taylor, R. W.; et al. Interferometric scattering microscopy reveals microsecond nanoscopic protein motion on a live cell membrane. Nat. Photonics 13, 480 (2019)

[2] Mazaheri, M.; et al. Label-Free Imaging and Tracking with Speckle-Free Interferometric Scattering Microscopy (in preparation)

[3] Kasaian, K.; et al. Long-Range High-Speed 3D Tracking via Interferometric Scattering Microscopy (in preparation)

BP 27.4 Thu 10:15 H 0112

An optogenetic method for the controlled release of single molecules — P Kashyap[1], S Bertelli[2], F Cao[3], Y Kostritskaia[4], F Blank[1], N Srikanth[3], C Schlack[1], R Saleppico[5], D Bierhuizen[1], X Liu[6], W Nickel[5], RE Campbell[6], A Plested[2], T Stauber[4], MJ Taylor[3], and •H Ewers[1] — [1]Freie Universität Berlin — [2]Humboldt-Universität zu Berlin — [3]MPI Infection Biology — [4]Medical School Hamburg — [5]Heidelberg University — [6]University of Alberta

We developed a system for optogenetic release of single molecules in live cells. We confined soluble and transmembrane proteins to the Golgi apparatus via a photocleavable protein and released them by short pulses of light. Our method allows for the controlled delivery of functional proteins to cytosol and plasma membrane in amounts compatible with single molecule imaging, greatly simplifying access to single molecule microscopy of any protein in live cells. Furthermore, we could reconstitute cellular functions such as ion conductance by delivering BK and VRAC ion channels to the plasma membrane. Finally, we could induce NF-kB signaling in T-Lymphoblasts stimulated by IL-1 by controlled release of a signaling protein that had been knocked-out in the same cells. We observed light induced formation of functional inflammatory signaling complexes that could trigger IKK phosphorylation in single cells. We thus developed an optogenetic method for the reconstitution and investigation of cellular function at the single molecule level.

BP 27.5 Thu 10:30 H 0112

A single-molecule perspective on the RNA interactions of a Ser-Arg-rich splicing factor — •Marie Synakewicz[1], Sarah Habeler[1], Steffen Winkler[1], Lucia Franchini[1], Héloïse Bürgisser[2], Antoine Cléry[1], Frédéric Allain[2], Nina Hartrampf[1], and Benjamin Schuler[1] — [1]University of Zurich, Zürich, Switzerland — [2]ETH Zurich, Zürich, Switzerland

Boundaries between coding and non-coding regions within mRNAs are recognised by serine-arginine-rich splicing factors (SRSFs) that contain one or two structured RNA recognition motifs (RRMs) and a long intrinsically disordered domain consisting of many Arg-Ser repeats. Extensive phosphorylation of RS domains modulates SRSF conformation, cellular localisation and function. Using single-molecule techniques, we aim to understand the molecular detail of the SRSF1-RNA interaction, and how this is regulated by the RS domain and its phosphorylation pattern. We characterised the interaction of full-length SRSF1, the RRMs and the RS domain with ssRNAs using single-molecule Förster Resonance Energy Transfer (smFRET), before showing that an increase in phosphorylation of the RS domain correlates with a decrease in affinity. More recently, we started to explore protein-RNA interactions in the context of a natural pre-mRNA construct. Using both smFRET and force spectroscopy we show that the conformational ensemble of the pre-mRNA consists of more than one structure, and that these are modulated by protein binding. Our results provide new insights into how SRSF1 can bind and modulate RNA structure, and therefore its capacity to regulate many cellular processes.

BP 27.6 Thu 10:45 H 0112

FRET-guided integrative modelling of (ribo-)nucleic acids — Fabio D. Steffen[1], Felix Erichson[2], and •Richard Börner[2] — [1]University of Zurich, Zurich, Switzerland — [2]Laserinstitut Hochschule Mittweida, Mittweida University of Applied Sciences, Mittweida, Germany

The functional diversity of RNA is encoded in their innate conformational heterogeneity. The combination of single-molecule spectroscopy and computational modeling offers new opportunities to map structural transitions within ribonucleic acid ensembles. Here, we describe a framework to harmonize single-molecule FRET measurements with molecular dynamics simulations and *de novo* structure prediction.

Using either all-atom or implicit fluorophore modeling we recreate FRET experiments in silico, visualize the underlying structural dynamics and quantify the simulated reaction coordinates. Using multiple accessible-contact volumes (multi-ACV) as a post-hoc scoring method for fragment-assembly in Rosetta FarFar2, we demonstrate that FRET effectively refines *de novo* RNA structure prediction without the need of explicit dye labeling *in silico*. We benchmark our FRET-assisted modeling approach on double-labeled DNA strands and validate it against an intrinsically dynamic Mn(II)-binding riboswitch and a Mg(II)-sensitif ribosomal RNA tertiary contact. We show that already one FRET coordinate, i. e., describing the assembly of a four-way junction and the GAAA binding to a kissing loop, allows to recapitulate the global fold of both, the riboswitch and the tertiary contact, and to significantly reduce the *de novo* generated structure ensemble.

15 min. break

Invited Talk BP 27.7 Thu 11:15 H 0112

Integrative dynamic structural biology with multi-modal fluorescence spectroscopy and nanoscopy: From single molecules to live cells — •Claus Seidel — Heinrich-Heine-University Düsseldorf, Germany

Multimodal fluorescence spectroscopy and microscopy with multiparameter detection provide rich insights on biomolecular systems under ambient / live cell conditions, including spatial, structural and kinetic information. In a comparative single-molecule study, we assessed the accuracy of Förster Resonance Energy Transfer (FRET) measurements. We studied two protein systems with distinct conformational changes and dynamics and obtained an interdye distance precision of smaller than 2 Å and accuracy of smaller than 5 Å. Considering cellular studies, we introduced a framework for quantitative high throughput FRET image spectroscopy. We measured the time-evolution of pairwise homo- or hetero-interactions of the Guanylate binding proteins and the membrane receptor CD95 in live cells with 0.8% fraction precision. In this way, the next level of complexity is achieved by linking structural dynamics of biomolecules with their cellular function and localization.

BP 27.8 Thu 11:45 H 0112

Cavity-enhanced ultrafast sensing of single nanosystems — •Shalom Palkhivala, Larissa Kohler, and David Hunger — Karlsruhe Institute of Technology, Karlsruhe, Germany

The investigation of single unlabelled nanosystems is of interest in branches of science such as biophysics and chemistry, where sensors are needed which can detect nanosystems in aqueous environments. We demonstrate an open-access optofluidic platform for the high-speed label-free sensing of nanoparticles in aqueous suspension. The heart of the sensor is a fibre-based Fabry-Perot microcavity with high finesse (5×10^4 in water) integrated into a microfluidic system. By monitoring the cavity resonance as the optical field interacts with a nanoparticle, the particle can be detected and characterised. We have demonstrated three-dimensional tracking of a single diffusing nanoparticle by measuring the resonance frequency shifts of several transverse modes [1]. Now, our cavity-locked detection scheme allows measurement of fast nanoparticle dynamics with a temporal resolution ($\sim$ 10 ns) orders of magnitude better than most other techniques. Additionally, orthogonal polarisation eigenmodes of the cavity are interrogated to yield orientational information of anisotropic particles. Thus, the rotation of single nanorods 20 nm long could be tracked with high measurement bandwidth, and the diffusion dynamics used to determine the dimensions of individual nanorods. We shall report progress towards using our sensor to investigate the dynamics of biological nanosystems, such as the folding of DNA "origami".

[1] Kohler, L. *et al.* Nat Commun **12**, 6385 (2021).

BP 27.9 Thu 12:00 H 0112

Residue Size Dependency of the Geminate Recombination Dynamics of the Biologically Relevant Disulfide Moiety after UV-cleavage investigated by TRXAS — •Jessica Harich — Institute of Nanostructure and Solid State Physics, University of Hamburg and Center for Free-Electron Laser Science, Germany

The tertiary structure of proteins is stabilized by disulfide bonds formed from two spatially adjacent L-cysteinyl residues. These disulfide bridges are prone to UV radiation damage with potentially adverse effects. We employ time resolved X-ray absorption spectroscopy (TRXAS) to observe the UV photochemistry of the natural amino acid dimer L-cystine and the tripeptide Glutathione disulfide in aqueous solution to understand the photochemistry under physiological conditions. Furthermore, we have first exciting insights into the UV-photochemistry of the disulfide bridges within the protein hen egg white Lysozyme.

We find that upon UV irradiation, apliphatic disulfides immediately undergo S-S bond cleavage, leading to the formation of two identical thiyl radicals, followed by fast geminate recombination indicating a very effective recombination process for thiyl radicals to the ground state. This process is only possible in condensed phases and its speed increases with chain length. Our results show that L-cystine already captures the essence of the ultrafast photochemistry of the disulfide bridge, but that the size of the residue adjacent to the disulfide bonds has a strong influence on the immediate recombination dynamics of the photoproducts.

BP 27.10 Thu 12:15 H 0112

Direct imaging of single RNAs — •Shengpeng Huang[1], Klaus Kern[1,2], and Kelvin Anggara[1] — [1]Max Planck Institute for Solid State Research — [2]Institute de Physique, École Polytechnique Fédérale de Lausanne

Ribonucleic acid (RNA) plays key roles in many biological processes, including gene expression, protein synthesis, chemical catalysis, and cellular regulation. Despite its ubiquity, understanding flexible RNA structures remain challenging with ensemble-averaged methods, such as X-ray crystallography, cryo electron microscopy, and nuclear magnetic resonance.

We confront this problem by direct imaging of RNAs deposited on surfaces, which offers an interesting possibility to determine directly the RNA sequence and its consequent three-dimensional structures. We employ the Electrospray Ion Beam Deposition (ESIBD) technique to transfer intact RNA molecules in a solution onto a surface in vacuo, which are subsequently imaged by Scanning Tunnelling Microscopy (STM). Using our ESIBD+STM approach, we have successfully deposited and imaged single chains of intact 60-mer RNA, which allows individual RNA chains to be structurally characterized at the single nucleotide level. Single molecule imaging of RNA presents a new approach to structurally determine many interesting post-translational modifications (PTMs) of RNAs with important biological roles.

BP 27.11 Thu 12:30 H 0112

Structure-Mechanics Relationships of Heterodimeric Coiled Coils — Zeynep Atris[1], Anna-Maria Tsirigoni[1], Melis Goktas[1], Patricia Lopez Garcia[1], Russell J. Wilson[1,2], Angelo Valleriani[1], Ana Vila Verde[3], and •Kerstin G. Blank[1,2] — [1]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany — [2]Johannes Kepler University, Linz, Austria — [3]University of Duisburg-Essen, Duisburg, Germany

Coiled coil (CC) structural motifs are found in diverse array of different proteins. Consisting of self-assembled alpha-helices that create helical superstructures, they serve as key elements of cytoskeletal and extracellular matrix proteins. Despite their widespread occurrence as mechanical building blocks, the fundamental structural factors governing their molecular mechanical properties have remained largely elusive.

We are applying AFM-based single molecule force spectroscopy and steered molecular dynamics simulations to determine the structure-to-mechanics relationship of de novo designed, synthetic CCs. When comparing heterodimeric CCs of varying length and sequence, our findings reveal that higher thermodynamic and kinetic stability does not always correlate with higher rupture forces within the range of AFM-accessible loading rates. We further observe that a single sequence can exhibit diverse mechanical stabilities under different loading geometries. This knowledge is now utilized for the development of a library of CC-based mechanoresponsive hydrogel crosslinks for tissue engineering applications.

BP 27.12 Thu 12:45 H 0112

Visualizing and quantifying biomolecular interactions with fluorescence optical tweezers. — •Roman Renger, Philipp Rauch, and Nicholas Luzzietti — LUMICKS, Amsterdam, The Netherlands

Biological processes involving proteins interacting with nucleic acids, membranes or cytoskeletal filaments are key to cell metabolism and hence to life in general. Detailed insights into these processes provide essential information for understanding the molecular basis of physiology and pathological conditions. The next scientific breakthrough

consists in direct, real-time observations and measurements of the most fundamental mechanisms and interactions involved in biology. Modern correlative single-molecule technologies offer a powerful opportunity to meet these challenges and to study dynamic protein function and activity in real-time and at unprecedented resolution. Here, we present our efforts to enable discoveries in biology and biophysics by combining optical tweezers with correlative fluorescence microscopy and advanced microfluidics. Our C-Trap allows to observe biomolecules while simultaneously measuring and controlling the generated forces and exposing the biomolecular system to different experimental conditions. We present examples in which our technology has enhanced the understanding of basic biological and biophysical phenomena, ranging from DNA repair to proteins dynamics to intracellular organization. Furthermore, we demonstrate how advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument for tackling biophysical questions.