BP 7: Single Molecule Biophysics

Time: Monday 15:00–17:00 Location: H44

Invited Talk BP 7.1 Mon 15:00 H44

Single-molecule dynamic structural biology with Graphene Energy Transfer — •Philip Tinnefeld[1], Alan Szalai[1], Giovanni Ferrari[1], Lars Richter[1], Ingrid Tessmer[2], Andres Vera-Gomez[1], and Izabela Kaminska[1] — [1]Chemistry Department and Center for NanoScience, Ludwig-Maximilians-Universität München — [2]Rudolf Virchow Center, University of Würzburg, Würzburg

Obtaining structural information from single molecules is commonly associated with Fluorescence Resonance Energy Transfer that typically yields one distance between a fluorescent donor and an acceptor. Energy transfer to graphene with graphene-on-glass coverslips can extend the dynamic range to more than 30 nm. Based on the discovery that DNA can be placed vertically on graphene, we developed GETvNA (graphene energy transfer with vertical nucleic acids) that enables Angstrom precise visualization of DNA conformations and protein-DNA complexes. We envision that the alignment of DNA will additionally make it amenable to combined energy transfer and super-resolution interrogation for dynamic structural biology.

BP 7.2 Mon 15:30 H44

Doubling the resolution of fluorescence-lifetime single-molecule localization microscopy with image scanning microscopy — •Niels Radmacher[1], Oleksii Nevskyi[1], José Ignacio Gallea[1], Jan Christoph Thiele[2], Ingo Gregor[1], Silvio O. Rizzoli[3,4], and Jörg Enderlein[1,4] — [1]Third Institute of Physics, Georg August University, Göttingen, Germany — [2]Department of Chemistry, University of Oxford, Oxford, UK — [3]Department of Neuro- and Sensory Physiology, University Medical Center Göttingen, Göttingen, Germany — [4]Cluster of Excellence - Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells (MBExC),Göttingen, Germany

In this study, we integrate a single-photon detector array into a confocal laser scanning microscope, enabling the combination of fluorescence-lifetime single-molecule localization microscopy with image scanning microscopy. This unique combination delivers a twofold improvement in lateral localization accuracy for single-molecule localization microscopy (SMLM) and maintains its simplicity. Moreover, the addition of lifetime information from our confocal laser scanning microscope eliminates chromatic aberration, particularly crucial for achieving few-nanometre resolution in SMLM. Our approach is named fluorescence-lifetime image scanning microscopy iSMLM. And is demonstrated through dSTORM and DNA PAINT experiments on fluorescently labelled cells, showcasing both resolution enhancement and fluorescence-lifetime multiplexing capabilities.

BP 7.3 Mon 15:45 H44

Two-color single-molecule coincidence detection for the analysis of biological processes and high-affinity bi-molecular binding — •Benno Schedler[1], Olessya Yuknovets[1], Alida Meyer[1], Lennart Lindner[1], and Jörg Fitter[1,2] — [1]RWTH Aachen University, I. Physikalisches Institut (IA), Aachen, Germany — [2]FZ Jülich, ER-C-3, Jülich, Germany

Life on the molecular scale is based on a versatile interplay of biomolecules, a feature that is relevant for the formation of macromolecular complexes. Fluorescence based two-color coincidence detection is widely used to characterize molecular binding and was recently improved by a brightness-gated version which gives more accurate results [1]. We developed and established protocols which make use of coincidence detection to quantify binding fractions between interaction partners labeled with fluorescence dyes of different colors. Since the applied technique is intrinsically related to single molecule detection, the concentration of diffusing molecules for confocal detection is typically in the low pico-molar regime. This makes the approach a powerful tool for determining bi-molecular binding affinities, in terms of KD-values, in this regime. By measuring the affinity at different temperatures, we were able to determine thermodynamic parameters of the binding interaction. The results show that the ultra-tight binding is dominated by entropic contributions [2].

References:

[1] Höfig et al. Communication Biology 2019 2, 459

[2] Schedler et.al. Int. J. Mol.Sci 2023 24, 16379

15 min. break

BP 7.4 Mon 16:15 H44

Maximizing Flavor: Leveraging Nano-biophysical Methods in Food Perception and Formulation Research — •Melanie Koehler — Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Lise Meitner-Straße 34, 85354 Freising, Germany — TUM Junior Fellow at the Chair of Nutritional Systems Biology, Technical University of Munich, Lise-Meitner-Straße 34, 85354 Freising, Germany

The food industry faces the challenge of creating healthier products with less salt, sugar, fat, and calories while maintaining flavor and consumer satisfaction. Flavor perception, influenced by taste, smell, texture, and individual factors, requires a deeper understanding to drive innovation. This research highlights the use of nano-biophysical techniques, particularly bio atomic force microscopy (AFM), to explore taste and texture at the molecular level. AFM provides nanoscale insights into food components' interactions with sensory receptors (taste- and mechanoreceptors), and their role in flavor release. For instance, AFM revealed the binding of a bitter peptide (VAPFPEVF) to its receptor (TAS2R16) without triggering downstream signaling. It also sheds light on oral texture perception, which remains underexplored at the biomolecular level [1]. By combining AFM with biochemical assays, molecular simulations, and human sensory evaluations, this research bridges objective measurements and subjective flavor experiences. These findings offer new approaches to designing healthier, sensory-appealing foods, addressing critical health and nutrition challenges. [1] Koehler, M, et al. Nature Food (2024): 1-7.

BP 7.5 Mon 16:30 H44

Label-Free Photothermal Infrared Correlation Spectroscopy — •Arthur Markus Anton and Frank Cichos — Leipzig University, Peter Debye Institute for Soft Matter Physics, Linnéstr. 5, 04103 Leipzig

Correlation spectroscopy is an indispensable method in modern life science. It allows for retrieving analyte properties in solution like it's concentration or diffusion coefficient, and thus enables to calculate the hydrodynamic radius of proteins or the viscosity of membranes, for instance. Common techniques are based on fluorescence or scattering, such as fluorescence correlation spectroscopy or dynamic light scattering respectively, and therefore need fluorescent labeling of analytes or lack of molecular specificity.

In this contribution we present a novel spectroscopic correlation technique on the basis of pumping with IR light but probing by means of visible light. The absorption of IR light is highly specific and allows us for *label-free* addressing of specific vibrational modes within the analyte molecules via the particular pump wavelength. Upon absorption, energy is dissipated and transferred into heat which alters the refractive index of the medium surrounding the absorbing species. This *transient* refractive index change is then probed by means of visible light providing a similar probe focal volume as conventional correlation spectroscopy techniques. Consequently, on the basis of IR pumping and visible probing we calculate the analyte's concentration and diffusion coefficient.

BP 7.6 Mon 16:45 H44

Single-protein optical holography — •Jan Christoph Thiele[1,2], Emanuel Pfitzner[1,2], and Philipp Kukura[1,2] — [1]Kavli Institute for Nanoscience Discovery, University of Oxford, UK — [2]Department of Chemistry, University of Oxford, UK

Light scattering by nanoscale objects is a fundamental physical property defined by their scattering cross-section and thus polarizability. Over the past decade, a number of studies have demonstrated single-molecule sensitivity by imaging the interference between scattering from the object of interest and a reference field. This approach has enabled mass measurement of single biomolecules in solution owing to the linear scaling of image contrast with molecular polarizability. Nevertheless, all implementations so far are based on a common-path interferometer and cannot separate and independently tune the reference and scattered light fields, thereby prohibiting access to the rich toolbox available to holographic imaging. Here we demonstrate comparable sensitivity using a non-common-path geometry based on a dark-

field scattering microscope, similar to a Mach-Zehnder interferometer. We separate the scattering and reference light into four parallel, inherently phase-stable detection channels, delivering a five orders of magnitude boost in sensitivity in terms of scattering cross-section over state-of-the-art holographic methods. We demonstrate the detection, resolution and mass measurement of single proteins with mass below 100 kDa. Separate amplitude and phase measurements also yield direct information on sample identity and experimental determination of the polarizability of single biomolecules.