BP 26: Single molecules biophysics

Time: Thursday 15:00–16:00 Location: H4

Invited Talk BP 26.1 Thu 15:00 H4

Understanding molecular machines by single-molecule FRET — •Thorsten Hugel — Institute of Physical Chemistry, livMatS and CIBSS, University of Freiburg, Germany

Many molecular machines associate and dissociate dynamically and/or alternate dynamically between multiple conformations. Common techniques are not ideal for studying such dynamics on relevant time scales from sub-micro-seconds to several hours. This dynamic information, ideally in and out of equilibrium, is crucial for a thorough understanding of the machines' function.

We have used networks of distance distributions obtained with single molecule FRET to simultaneously quantify large global conformational changes (seconds) and local dynamics (microseconds to milliseconds) in the molecular chaperone and heat shock protein Hsp90. The data reveal a state-specific suppression of the sub-millisecond fluctuations by dynamic Hsp90-substrate interactions, enabling an additional (orthogonal) regulation mechanism. The fundamental precision and accuracy of single-molecule FRET measurements as well as multi-color single molecule FRET will also be discussed.

In addition, we have developed a plasmon-ruler based single-molecule approach to study the conformational dynamics of Hsp90 over 24 hours at video rate. This unprecedented dynamic bandwidth reveals states with surprisingly long dwell times of many minutes. To be discussed are the impact of these findings on our understanding of conformational heterogeneity among proteins, protein denaturation, ergodic behavior, and non-Markovian dynamics (memory effects).

BP 26.2 Thu 15:30 H4

AFM-based Single-Molecule Force Spectroscopy on the Streptavidin:Biotin Interaction — •Steffen M. Sedlak[1], Leonard C. Schendel[1], Katherine R. Erlich[1], Achim Löf[1], Rafael C. Bernardi[2], Magnus S. Bauer[1], Carleen Kluger[1], and Hermann E. Gaub[1] — [1]Department of Physics and Center for NanoScience, LMU Munich, Germany — [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA

The high-affinity interaction of the small molecule biotin with the tetrameric protein streptavidin (SA) is a widely applied tool for detection, labeling and immobilization of molecules. We study single biotin:SA interactions under force using AFM-based single-molecule force-spectroscopy (SMFS) and steered Molecular Dynamics (sMD) simulations. Probing monovalent SA in various specific tethering geometries, we investigated how the mechanical stability of the biotin:SA interaction depends on the force loading geometry and revealed the underlying molecular mechanism. We made use of the different unbinding forces to realize a protein-based bottom-up nanoscale assembly of single fluorescent molecules by single-molecule cut-and-paste; a unique approach that enables spatially controlled arrangements of diverse molecules into a single ensemble. We also studied SA of different valencies and distinguished unbinding forces of biotin from different SA subunits in AFM-based SMFS. sMD allowed to understand the force-propagation pathways through the SA tetramer. Identifying a long-lived tethering geometry, we can reliably measure single molecules at comparably high constant forces for many hours in magnetic tweezers.

BP 26.3 Thu 15:45 H4

Angstrom precision distance measurements in dynamic protein structures with single-molecule FRET — •Christian Gebhardt, Rebecca Mächtel, Niels Zijlstra, and Thorben Cordes — LMU München, Faculty of Biology, Großhaderner Str. 2-4, 82152 Planegg, Germany

Single-molecule Förster resonance energy transfer (smFRET) has evolved towards a mature toolkit for the study of distances, structures and dynamics of biomolecules in a physiologically relevant context in vitro and in vivo. There is, however, no generally accepted way to derive and use quantitative distance information from the FRET-ruler to derive structural models or constraints in the protein data base. Hellenkamp et al. (Nat. Methods, 2018) recently presented a quantitative smFRET study of oligonucleotide ruler structures that revealed high precision, accuracy and reproducibility of FRET-derived distances in a worldwide comparative study of 20 labs with a distance uncertainty below 6 angstrom. While this establishes smFRET as a suitable technique for accurate distance measurements of static biological reference structures, we raise the question if smFRET is applicable for proteins with dynamic conformational motions or allosteric modulations of protein structure by an effector. Proteins are more challenging targets for site-specific fluorophore labelling. We identified a model system to benchmark FRET-derived distance uncertainties in proteins for situations of (i) stochastic labelling and (ii) allosteric and dynamic modulation of the structure and show similar angstrom precision comparable to DNA.