BP 28: Single Molecule Biophysics

Time: Thursday 9:30–13:00 Location: H 1058

BP 28.1 Thu 9:30 H 1058

Cell free protein synthesis systems and single molecule fluorescence studies: a perfect marriage — Alexandros Katranidis[1], Mayuri Sadoine[1], Noemie Kempf[1], Michael Gerrits[2], Michele Cerminara[1], and •Jörg Fitter[1,3] — [1]Research Centre Juelich, ICS-5, Juelich, Germany — [2]TU Berlin, Biocatalysis Group, Department of Chemistry, Berlin, Germany — [3]RWTH Aachen University, I. Physikalisches Institut (IA), AG Biophysik, Aachen, Germany

Protein synthesis is a fundamental cellular process, by which ribosomes decode genetic information and convert it into an amino acid sequence. This highly complex process does not necessarily require cell integrity, but can also proceed in so called cell-free protein systems. This opens the door for comprehensive studies to obtain a deeper understanding of individual steps of the translation cycle and of the folding of de novo synthesized proteins. The use of cell-free protein synthesis (CFPS) systems allowed us to watch some of these essential steps in real time and on single molecule level [1,2]. On the other hand the open nature of the CFPS system allows the production of tailor-made protein samples, perfectly suited for single molecule Förster resonance energy transfer (smFRET) studies [3]. Examples from both above mentioned topics will be presented and demonstrate the strength of combining CFPS with single molecule fluorescence studies.

[1] A. Katranidis et al., Angewandte Chemie Int. Edit., 48, 1758-1761, (2009); [2] N. Kempf et al., Sci. Rep., 7, 46753, (2017); [3] M. Sadoine et al., Anal. Chem., 89, 11278-11285, (2017)

BP 28.2 Thu 9:45 H 1058

Coiled Coils as structural building blocks: A sequence-based approach towards tuning Coiled Coil mechanics — •Patricia López-García, Melis Göktas, and Kerstin G. Blank — Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany

The natural abundance of coiled coil (CC) motifs in cytoskeleton and extracellular matrix proteins suggests that CCs play an important role as passive (structural) and active (regulatory) mechanical building blocks. It is well established that modifications in CC sequence, e.g. in hydrophobic core or solvent-exposed residues, are responsible for the thermodynamic stability of CCs; however, nothing is known about how these factors affect CC mechanics.

With the goal of shedding light on the sequence-structure-mechanics relationship of CCs we have chosen thermodynamically well-characterized sequences and analyzed their mechanical stability using single molecule force spectroscopy, applying force parallel to the helical axis in the shear geometry. Modifications in the hydrophobic core or the helix propensity alter the binding potential with different outcomes: a less tightly packed hydrophobic core increases the potential width without significantly affecting the barrier height (koff). In contrast, a reduced helix propensity decreases both potential width and barrier height. Our goal is to use this information for developing a library of mechanically characterized CCs that can be applied as calibrated building blocks for a wide range of applications: from molecular force sensors to mechanosensitive material crosslinks in protein nanostructures and synthetic ECM mimics.

BP 28.3 Thu 10:00 H 1058

Tuneable reversibility in force probe simulations — •Stefan Jaschonek and Gregor Diezemann — Institut für Physikalische Chemie, Duesbergweg 10-14, 55128 Mainz

In this talk a detailed study of the dependence of force probe molecular dynamics (FPMD) simulations on the pulling parameters is presented. As a model system, the well studied calix[4]arene catenane dimer was chosen. This system consists of two "cups", the calixarene structure, which are mechanically locked by aliphatic loops of tuneable length, realized by a catenane structure. The dimer shows reversible rebinding meaning that the opening and the rebinding transition can be monitored. Due to the tuneable loop length it is possible to gain full control over the energy landscape. The kinetics of the system can be understood in terms of a two state model for shorter loops (≤ 14 CH_2 units) and a three state model for longer loops (≥ 17 CH_2 units).

The impact of a systematical variation of the pulling parameters, the pulling velocity V and the stiffness K, of the externally applied potential and the loop length of the system are discussed. Furthermore the characteristic unbinding and rebinding forces are analyzed and the kinetic rates are extracted.

BP 28.4 Thu 10:15 H 1058

Non-Markovian bond kinetics and its application in dynamic force spectroscopy — •Jakob Tómas Bullerjahn[1,2], Sebastian Sturm[2], and Klaus Kroy[2] — [1]Max-Planck-Institut für Biophysik, Frankfurt am Main, Germany — [2]Universität Leipzig, Institut für theoretische Physik, Leipzig, Germany

Single-molecule force spectroscopy data are conventionally analyzed using a schematic model, wherein a molecular bond is modeled as a virtual particle diffusing in a one-dimensional free-energy landscape. This simplistic but efficient approach is unable to account for the "anomalous" bond-breaking kinetics increasingly observed in high-speed force spectroscopy experiments and simulations, such as a non-exponential distribution of bond lifetimes under constant load. In the traditional framework, the only remedy has been to postulate a multitude of intermediate states. Here, we introduce a complementary approach, namely a rigorous extension of the one-dimensional standard theory that accounts for the transient dynamics of a generic set of coupled degrees of freedom. These "hidden modes" affect the reaction dynamics in various ways, depending on their relaxation spectrum. We find exact analytical expressions for pertinent experimental observables, such as the mean rupture force and the rupture force distribution, in two asymptotic limits. They become unconditionally exact at high loading rates, thus providing us with a microscopically consistent theory of rapid force spectroscopy that avoids the usual Markov assumption.

Invited Talk BP 28.5 Thu 10:30 H 1058

Multiplexed Magnetic Tweezers: From DNA Mechanics to Retroviral Integration — •Jan Lipfert, Franziska Kriegel, Willem Vanderlinden, and Philipp Walker — Department of Physics and Center for Nanoscience, LMU Munich, Germany

Magnetic tweezers are a powerful tool to probe single DNA molecules and their complexes with proteins under controlled forces and torques at the single molecule level. Using a parallelized version of magnetic tweezers that can measure torque directly, we have carried out high-precision torque measurements of DNA mechanics. Our results indicate that the intrinsic torsional stiffness does not change with mono- or divalent ion concentration and is approximately independent of temperature, for temperatures well below the melting temperature. Quantitative comparison of high-resolution single molecules measurements to coarse-grained simulations of DNA mechanics shows that taking into account the anisotropy of DNA and introducing a non-zero twist-bend coupling significantly improves agreement with torque measurements. In addition, we demonstrate that all-atom molecular dynamics simulations correctly predict the temperature-dependence of DNA twist and of DNA torsional stiffness, if the most recent force fields are used. Going beyond bare DNA, we have developed a magnetic tweezers assay to follow retroviral integration in real time, revealing several critical steps along the integration free energy landscape. In particular, we find an ultra stable strand transfer complex that suggests the role of a resolving factor in vivo.

15 min. break

BP 28.6 Thu 11:15 H 1058

Interaction of DNA structures with ectoine: a molecular dynamics simulation study — •Ewa Anna Oprzeska-Zingrebe and Jens Smiatek — Institute for Computational Physics, University of Stuttgart, Stuttgart, Germany

In nature, the cellular environment of DNA composes not only of water and ions, but also of salts, lipids and other cosolutes, which can exert both stabilizing and destabilizing influence on the formation and existence of particular DNA higher-order forms. Among them, ectoine, known as osmoprotectant occurring naturally in halophilic bacteria and other microorganisms exposed on severe osmotic stress, turns out to be of particular importance. In our research, we investigate the behavior of a short 7-bp DNA oligonucleotide with the sequence d(GCGAAGC) in both linear and folded form, as well as 24-bp B-DNA duplex in aqueous solution with various concentrations of ectoine. Our results demonstrate a DNA conformation-dependent binding behavior,

which allows us to rationalize the structure-stabilizing influence of ectoine.

BP 28.7 Thu 11:30 H 1058

Pressure effect on the conformational landscapes of a large loop DNA hairpin in the presence of osmolyte and salt — •Satyajit Patra[1], Vitor Schuabb[1], Irena Kiesel[1], Jim Marcel Knop[1], Rosario Oliva[2], and Roland Winter[1] — [1]Fakultät für Chemie und Chemische Biologie, TU Dortmund, Otto-Hahn-Str. 6 — [2]Department of chemical sciences, University of Naples Federico II, Via Cinita, 80126 Naples, Italy

The conformational landscapes of a large loop DNA hairpin model system has been investigated in absence and presence of salt and osmolytes at both ambient and extreme conditions (high hydrostatic pressure and high temperature) using primarily single molecule Förster resonance energy transfer (smFRET) technique. We use cationic salts (K^+, Mg^{2+}, Co^{3+}) and TMAO and urea as osmolytes. Introduction of pressure favors the open state (low FRET species) of this DNA hairpins thus facilitates the unfolding. Addition of the salt in the solution populates the high FRET species and counteract the pressure and temperature effect. The order of stabilizing effect of the salt against pressure and temperature follows the order $Co^{3+} > Mg^{2+} > K^+$. Introduction of urea and temperature favors the formation of intermediate state populations which is further supported by smFRET measurement under immobilized condition. This is indicating that the free energy landscapes of this large loop DNA hairpin is actually a rugged one. Further smFRET measurement under immobilized condition provides a deeper insights into the differential stabilization mechanism of salt and osmolytes.

BP 28.8 Thu 11:45 H 1058

DNA strand break yields by OH-radicals, low energy electrons and prehydrated electrons — •Marc Benjamin Hahn[1,2], Tihomir Solomun[2], and Heinz Sturm[2,3] — [1]Freie Universität Berlin — [2]Bundesanstalt für Materialforschung und -prüfung — [3]Technische Universität Berlin

Radiation damage to biomolecules such as DNA, is the reason to treat cancer *via* radiation therapy. The understanding of the molecular processes and the quantification of the underlying damaging mechanisms is necessary to develope more efficient irradiation protocols for cancer therapy. Thereby damage to DNA is of key interest due to its central role in reproduction and mutation. Due to the high amount of water in biological tissue, most of the damage is caused by the secondary particles which are produced by the interaction of ionizing radiation with water. Thereby a multitude of species are produced, e.g. kinetic low energy electrons, prehydrated electrons, OH-radicals and ions. The quantification of the contribution to DNA damage by the various species is of interest. Here we present an experimental approach to disentangle their relative DNA strand break yields. Plasmid DNA (*pUC19*) is irradiated in water with electrons under the presence of different scavengers. The presented preliminary results reveal the relative contributions of OH-radicals, low energy electrons and prehydrated electrons and their DNA single and double strand break yields.

BP 28.9 Thu 12:00 H 1058

Detection of nanoscale biological samples using Nanocapillaries — •Tobias Jäckering[1], Marco Radukic[2], Dario Anselmetti[1], and Martina Viefhues[1] — [1]Experimental Biophysics, Physics Faculty, Bielefeld University, Bielefeld, Germany — [2]Biotechnological Faculty, Bielefeld University, Bielefeld, Germany

Filamented borosilicate glass capillaries can be pulled down to 40 nm diameter nanopipettes with the ability of easy filling the nanopipette via capillary forces. These nanopipettes were used for the detection and analysis of nanometer sized biological analytes by monitoring the electric (ionic) current during translocation through the nanopipette. We applied either electrophoretic or hydrodynamic driving forces to investigate different types of analytes like DNA and adeno-associated viruses (AAV).

To differentiate the detected signal from the background it is essential to adjust the nanopore-size of the nanopipette to the respective analytes size. Additionally the respective buffer has a significant effect on the measurement. We will demonstrate that these nanoscopic coulter-counter experiments allow to distinguish various analytes like DNA and AAV.

BP 28.10 Thu 12:15 H 1058

Antenna-enhanced fluorescence correlation spectroscopy resolves calcium-mediated lipid-lipid-interactions — •Stephan Block[1,2], Srdjan S. Aćimović[1], Nils Odebo Länk[1], Mikael Käll[1], and Fredrik Höök[1] — [1]Chalmers University of Technology, Göteborg, Sweden — [2]Freie Universität Berlin, Berlin, Germany

Fluorescence correlation spectroscopy (FCS) has provided a wealth of information on the composition, structure, and dynamics of cell membranes. However, it has proved challenging to reach the spatial resolution required to resolve biophysical interactions. Herein, we form artificial cell membranes on dimeric, nanoplasmonic antennas, which shrink the FCS probe volume down to the 20 nm length-scale. By analysing fluorescence bursts from individual fluorescently tagged lipids moving through the antenna hot spots, we show that the confinement of the optical readout volume below the diffraction limit allows the temporal resolution of FCS to be increased by up to 3 orders of magnitude. Employing this high spatial and temporal resolution to probe diffusion dynamics of individual dye-conjugated lipids, we further show that lipid molecules diffuse either as single entities or as pairs in the presence of calcium ions. Removal of Ca2+ by addition of EDTA almost completely removes the complex contribution, in agreement with previous theoretical predications on the role of Ca2+ in mediating transient interactions between zwitterionic lipids. We envision that antenna-enhanced FCS will enable to resolve a broad range of challenging membrane biophysics questions, such as stimuli-induced lipid clustering and membrane protein dynamics.

BP 28.11 Thu 12:30 H 1058

Visualizing cellular secretion dynamics with single protein sensitivity — •Katharina König[1,2], André Gemeinhardt[1], Matthew P. McDonald[1], and Vahid Sandoghdar[1,2] — [1]Max Planck Institute for the Science of Light, Erlangen — [2]Friedrich Alexander University Erlangen-Nuremberg, Erlangen

Cellular secretion of proteins and exosomes into the extracellular environment is an essential mediator of critical biological mechanisms, including cell-to-cell communication, immunological response, targeted delivery, and differentiation. Here, we report a novel methodology that allows for the real-time detection and imaging of single unlabeled proteins and exosomes that are secreted from individual living cells. This is accomplished via interferometric detection of scattered light (iSCAT), and is first demonstrated with human B cells that are found to actively secrete IgG antibodies at a rate of ca. 100 molecules per second. Importantly, iSCAT signals can be measured at extremely high speeds (up to MHz for small nanoparticles), enabling the measurement of single cell secretion dynamics with sub-second temporal resolution and single protein sensitivity. Such experimental capabilities are unmatched by any contemporary proteomic method. We furthermore show the generality of the technique through the study of T cell cytokine secretion, Leishmania parasite exosome release, and single-cell lysate analysis. Our results establish iSCAT imaging as a powerful label-free tool for studying the real-time exchange between cells and their immediate environment with single protein sensitivity.

BP 28.12 Thu 12:45 H 1058

Reductive caging and photoactivation in single-molecule Förster resonance energy transfer experiments — •Atieh Aminian Jazi[1], Evelyn Ploetz[2], Muhamad Arizki[1], Christine Ziegler[4], Reinhard Krämer[4], and Thorben Cordes[1,3] — [1]Zernike Institute for Advanced Materials, Groningen, The Netherlands — [2]Department of Chemistry and CeNS, Ludwig Maximilians-Universität, Munich, Germany — [3]Department Biology I, Ludwig-Maximilians-Universität München, Germany — [4]Institute of Biophysics and Biophysical Chemistry, Universität Regensburg,Germany

Förster-resonance energy transfer(FRET), in combination with single-molecule detection, has become a powerful tool to investigate the structural dynamics of biomolecular systems. We used caging of fluorophores by reversible chemical deactivation of fluorescence.

The diffusing molecules can be reactivated by ultraviolet (UV) light. UV-reactivation allows retrieving both FRET-related distances and sorting of multiple intramolecular spices in solution-based smFRET. We employed caged FRET to investigate the structure of a membrane transporter BetP, as multi-subunit protein, and nucleic acids containing more than two fluorescent labels. The results revealed that chemical caging and photoactivation (uncaging) by UV light allows temporal uncoupling of convoluted fluorescence signals from multiple donors or acceptor molecules.Caged FRET also can be used in a further application to study the intermolecular details of low-affinity binding interactions with diffusion-based smFRET.