BP 24: Posters - Single Molecule Biophysics

Time: Monday 17:30–19:30 Location: Poster C

BP 24.1 Mon 17:30 Poster C

Knotting and Unknotting of a single protein with optical tweezers — •Fabian Ziegler[1], Nicole Lim[1], Soumit Mandal[2], Benjamin Pelz[1], Wei-Ping Ng[2], Sophie Jackson[2], and Matthias Rief[1] — [1]Physik-Department E22, TU München (Germany) — [2]University of Cambridge (UK)

Spontaneous folding of a polypeptide chain into a knotted structure remains one of the most puzzling and fascinating features of protein folding. However the observed kinetics are on the timescale of minutes and thus hard to reproduce with atomistic simulations yet.

Former studies could not distinguish between folding and knotting steps in the formation of the knotted native structure, as it has generally not been possible to control the topology of the unfolded state. We have overcome this problem with Single-molecule Force Spectroscopy by variation of pulling directions and can provide direct evidence that a threading event associated with knot formation significantly slows down folding of native UCH-L1, an important enzyme in the proteosomal degradation that has mutations linked to Parkinson's disease and has been identified as a target for treatment of Alzheimer's disease.

Our results highlight the complex nature of the folding of a knotted protein, and detect many additional intermediate structures. Mechanical stretching of knotted proteins is also important for understanding the possible implications of knots in proteins for cellular degradation. Our results might therefore indicate one possible answer to the often raised question about functions and reasons for knotted structures in proteins.

BP 24.2 Mon 17:30 Poster C

Direct observation of the intermolecular association of PGL-3, a liquid protein droplet component. — •Jose A. Morin[1,2], Shambaditya Saha[3], Anthony Hyman[3], and Stephan Grill[2,3] — [1]Max Plank Institute for the Physics of Complex Systems, Nöthnitzer Str. 38, 01187 Dresden, Germany — [2]Biotechnology Center, Technical University, Tatzberg 47, 01307 Dresden, Germany — [3]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany

P granules, the germ line determinants in C. elegans, are an excellent example of spatiotemporal cytoplasmatic organization. During the first cell division these non-membranous organelles undergo a liquid-liquid phase separation and become localized to the posterior half of the cell. It have been shown that PGL-3, a key component of P granules, is capable of phase separation by it self into a liquid phase in the test tube. In this work we address the molecular causes for liquid formation in protein systems. We have stablished a single molecule experimental assay to measure the interaction energy between individual PGL-3 proteins. Using the manipulation capabilities of our dual trap optical tweezers, a single molecule force spectroscopy technique, we place two PGL-3 coated beads into close proximity to accurately measure the interaction kinetics between a small number of PGL-3 proteins. Our data exhibit a rich kinetic behavior, where transient interactions can be clearly distinguished from the background and therefore the frequency and time duration of this interactions accessed. Moreover, a cooperative association between these proteins can be hinted.

BP 24.3 Mon 17:30 Poster C

Complex Folding Kinetics of the SAM-I Riboswitch Expression Platform Revealed by Single-molecule FRET and HMM Analysis — •Christoph Manz[1], Andrei Yu Kobitski[1], Bettina Keller[2], Ayan Samanta[3], Andres Jäschke[3], and Gerd Ulrich Nienhaus[1,4] — [1]Institute of Applied Physics, Karlsruhe Institute of Technology, Wolfgang-Gaede-Str. 1, 76131 Karlsruhe, Germany — [2]Institute of Chemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany — [3]Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany and Molecular Biotechnology, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany — [4]Department of Physics, University of Illinois at Urbana-Champaign, 1110 West Green Street, Urbana, Illinois 61801, USA

Isolated aptameric domains of riboswitches have been studied intensively, whereas detailed knowledge on interactions between the aptamer and the expression platform of entire riboswitches is still lacking. We have studied the structure and dynamics of a complete S-adenosylmethionine-I riboswitch (SAM-I RS) at different Mg^{2+} and ligand concentrations by using single-molecule Förster resonance energy transfer (smFRET). To observe conformational changes in real time, we performed Mg^{2+} and SAM titration experiments on freely diffusing and on surface-immobilized SAM-I RS, using various FRET-labeled constructs. Data were analyzed with a newly developed hidden Markov model and an optimization procedure for photon-based smFRET data, yielding a detailed folding pathway for the SAM-I RS.

BP 24.4 Mon 17:30 Poster C

Nanomechanics of Fluorescent DNA-Dyes on DNA investigated by Magnetic Tweezers — •Ying Wang, Andy Sischka, Volker Walhorn, Katja Tönsing, and Dario Anselmetti — Experimental Biophysics and Applied Nanoscience, Physics Department, Bielefeld University, Universitätsstrasse 25, 33615 Bielefeld, Germany

Fluorescent DNA-dyes are broadly used in many biotechnological applications for detecting and imaging DNA in cells and gels. Their specific and selective interaction alters the structural and nanomechanical properties of DNA and affects biological processes that are associated with it. Although interaction modes like intercalation and minor grove binding already have been identified, associated mechanic effects like local elongation, unwinding, and softening of the DNA often remain poorly understood. We used magnetic tweezers in order to quantitatively investigate the impact of three DNA-binding dyes (YOYO-1, DAPI and DRAQ5) in a concentration dependent manner. By extending and overwinding individual, torsionally constrained, nick-free dsDNA molecules, we measured the contour lengths and molecular forces which allow estimation of several thermodynamic and nanomechanical binding parameters. Whereas for YOYO-1 and DAPI the binding mechanisms could be allocated to bis-intercalation and minor groove binding, respectively, DRAQ5 exhibited both binding modes in a concentration dependent manner.

BP 24.5 Mon 17:30 Poster C

Construction of an Optical Trap for Single Molecule Measurements on Nucleosomes — •Andreas Weissl[1], Pascal Hauenstein[1], Philip Ketterer[1], Corinna Lieleg[2], Fabian Kilchherr[1], Jonas Funke[1], Philipp Korber[2], Henrik Dietz[1], and Matthias Rief[1] — [1]Physik Department, Technische Universität München, Am Coulombwall 4a, Garching bei München, Germany — [2]Adolf-Butenandt-Institute, University of Munich, Munich, Germany

The folding of nucleosome arrays to chromatin fibres and higher order structures plays a major regulatory role in processes related to DNA transcription and replication in eukaryotic cells. A fundamental understanding of this mechanism requires insight in the formation of chromatin fibres on the molecular level.

We report on the construction of an optical trap suitable to perform single molecule measurements on nucleosomes. To push force resolution to its limits we realised a intensity feedback control that stabilised the laser intensity 0.05%. To be able to measure the same molecule in different buffer conditions we built a multi channel microfluidic setup, where we are to quickly move to various channels with different buffers.

We use this setup in a Dumbbell assay to measure the stacking force of two single nucleosomes. To conjugate the beads with the nucleosomes DNA nanostructured handles are employed. Mainly for two reasons: First, the increased stiffness compared to dsDNA handles reduces the noise, especially at low forces. Second, due to the high control over DNA nanostructures, we are able to control the orientation of the nucleosome interaction with respect to the direction of the force applied.

BP 24.6 Mon 17:30 Poster C

Splitting of plasmid ds-DNA on ultrathin films of alkylamines on graphite — •Caroline Falk[1], Nikolai Severin[1], Lei Tang[2], Stefan Zauscher[2], and Jürgen P. Rabe[1] — [1]Department of Physics & IRIS Adlershof, Humboldt-Universität zu Berlin — [2]Mechanical Engineering and Materials Science, Duke University

DNA replication is an important process in the human body. Replication of double-stranded (ds)-DNA requires unwinding of the helical structure and local melting of ds-DNA into two single strands [1]. The exact mechanism of the initial melting is still unknown. DNA, when stretched in solution, overwinds and melts [2]. This was argued to

give insight onto the replication mechanism. It is difficult, however, to access the direct conformational changes during stretching in solution. Recent work demonstrated that this transition can be imaged with scanning force microscopy on a graphite surface coated with an alkylamine layer [3]. ds-DNA can be controlled by an amphiphilic layer, since the DNA conformation depends on the amphiphile concentration. In particular we analyzed different DNA lengths on the same surface, and we found that at a specific concentration of octadecylamine the ds-DNA plasmid ring splits into two single strands at one position. The splitting can be analyzed as a function of total plasmid length, ultrathin amphiphilic film and base pairs.

1. D.Coman, I.M.Russu, Journal of Biological Chemistry 280, 20216 (2005). 2. J.Adamcik, S.Tobenas, G.Di Santo, D.Klinov, G.Dietler, Langmuir, 25, 3159 (2009). 3. H.Liang, N. Severin, W.Zhuang, J.P.Rabe, submitted.