BP 6: Posters: DNA and DNA Enzymes

Time: Monday 17:15–20:00 Location: Poster B1

BP 6.1 Mon 17:15 Poster B1

Electrostatic effects in DNA cyclization and DNA looping by proteins — •Andrey Cherstvy — IFF-2, FZ Jülich, Germany

We calculate analytically the electrostatic energy of ring formation for highly charged DNA-like polyelectrolyte chain in the ground state [1]. We study how the degree of counterion condensation differs on small DNA rings as compared to Manning prediction for straight polyelectrolytes. We apply the model to analyze how cyclization factors of short DNAs are modified, as compared to Shimada-Yamakawa theory for neutral polymers. We also perform simple computer simulations to assess the free energy of end joining of fluctuating DNA at different salinities. We study also the looping of thin charged elastic filaments under applied torque and forces, using the elasticity theory equations [2]. We predict that larger twist rates are needed to create loops in charged cables as compared to neutral ones. We determine optimal shapes of charged loops at different salinities showing that at low salts more open loops are favored due to enhanced mutual repulsion of charges. This loop opening is consistent with results of recent computer simulations on formation of DNA loops by lac repressor.

[1] A. G. Cherstvy and R. Everaers, in preparation. [2] A. G. Cherstvy, submitted to JPCB.

BP 6.2 Mon 17:15 Poster B1

Information transfer and DNA microarrays — •Christian Trapp and Albrecht Ott — Biologische Experimentalphysik, Universität des Saarlandes, Saarbrücken

DNA microarrays take advantage of the molecular recognition of DNA hybridization. They consist of surface bound probe ssDNA, which will selectively bind to complementary target strands in solution. They are widely employed in biotechnological applications such as genome expression profiling in order to assess complex mixtures of DNA. In spite of their importance DNA hybridization on microarrays remains poorly understood. We have shown that the binding to DNA microarrays can be modeled well in simple cases, where the length of probe and targets match. Here we investigate the binding of longer targets to microarrays, which may hybridize to the probes forming loops. We systematically vary loop position and loop size and check, if the result can be reproduced with simple theoretical models at thermal equilibrium. We discuss the influence of loops in terms of competitive binding. The ultimate goal is to explore the physical limits of information that can be conveyed through complex mixtures of DNA.

BP 6.3 Mon 17:15 Poster B1

High resolution optical tweezers for study of eukaryotic transcription — •Noemi Maria Porcellato, Adam Muschielok, and Jens Michaelis — Dep. für Chemie und Biochemie LMU München, Munich, Germany

For the single-molecule analysis of enzymes moving on DNA, for instance eukaryotic RNA polymerase II (Pol II), we have built a high resolution dual-trap optical tweezers apparatus. The main aim is to achieve very high spatial resolution (down to the level of single basepairs) and low drift. Since both traps are created by the same laser and the optical path where the beams are separated is minimized, drift is essentially eliminated. Together with sound isolation and enclosure of the instrument we achieve high spatial resolution shown in test experiments with DNA tethers. In order to efficiently load beads into the traps and to control buffer conditions during the experiments we use a custom built 5 channel microfluidic flow system. We discuss general design considerations, show calibration of the trap stiffness, first test measurements and preliminary Pol II transcription data.

BP 6.4 Mon 17:15 Poster B1

single-molecule force spectroscopy on phoB-DNA complexes — •Michael Birlo[1], Katrin Wollschläger[2], Rainer Eckel[3], Norbert Sewald[4], and Dario Anselmetti[5] — [1]Department of Physics Bielefeld University 33615 Bielefeld (Germany) — [2]Department of Chemistry Bielefeld University 33615 Bielefeld (Germany) — [3]Department of Physics Bielefeld University 33615 Bielefeld (Germany) — [4]Department of Chemistry Bielefeld University 33615 Bielefeld (Germany) — [5]Department of Physics Bielefeld University 33615 Bielefeld (Germany)

Interactions between proteins and DNA are essential for the regulation of cellular processes in all living organisms. In this context, it is of special interest to investigate the sequence-specific molecular recognition between transcription factors and their cognate DNA sequences. As a model system, peptide and protein epitopes of the DNA-binding domain (DBD) of the transcription factor PhoB from Escherichia coli are analyzed with respect to DNA binding at the single-molecule level. Quantitative AFM-DFS analysis proves the specificity of the interaction and yields force-related properties and kinetic data, such as thermal dissociation rate constants. An alanine scan for strategic residues in both peptide and protein sequences is performed to reveal the contributions of single amino acid residues to the molecular-recognition process.

BP 6.5 Mon 17:15 Poster B1

Binding Kinetics of Bisintercalator Triostin A Measurements with Optical Tweezers — •Andy Sischka[1], Christoph Kleimann[2], André Spiering[1], Katja Tönsing[1], Norbert Sewald[3], Ulf Diedrichsen[4], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanosciences, Bielefeld University, 33615 Bielefeld, Germany — [2]Institut für Bio- und Nanosysteme (IBN-3), Forschungszentrum Jülich, 52425 Jülich, Germany — [3]Organic and Bioorganic Chemistry, Bielefeld University, 33615 Bielefeld, Germany — [4]Organic and Biomolecular Chemistry, Göttingen University, 37077 Göttingen, Germany

We present single molecule binding studies where the intercalative binding kinetics of Triostin A to λ-DNA was investigated by measuring the force/extension response with our optical tweezers system [1]. These curves were analyzed based on a method for monointercalators that was extended to bisintercalators. Our measurements with Triostin A showed non-equilibrium phenomena, resulting in large hysteresis effects during a fast stretching/relaxation cycle, whereas at slow velocities, the system reaches an equilibrium state and the hysteresis vanishes. Subsequent binding analysis reveals an exponential dependence of the association constant on the external force as well as a decreasing binding site size. To explain the high-force binding site size, a new model for bisintercalation of Triostin A is proposed, where the deformation of the Triostin A binding site could thereby repeal the neighbor exclusion principle, leading to closer packaging of Triostin A.

[1] Ch. Kleimann, A. Sischka et al., Biophys.J 97, 2780-2784 (2009)

BP 6.6 Mon 17:15 Poster B1

Optical Tweezers Measurements of Threading Individual DNA and DNA-Ligand-Complexes through Solid-State Nanopores — •Andre Spiering[1], Andy Sischka[1], Tanja Plötz[1], Christoph Kleimann[2], Katja Tönsing[1], Ina Seuffert[3], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanosciences, Bielefeld University, 33615 Bielefeld, Germany — [2]Institut für Bio- und Nanosysteme (IBN-3), Forschungszentrum Jülich, 52425 Jülich, Germany — [3]Bioenergetics, Institute of Chemistry, Technische Universität Berlin, 10623 Berlin, Germany

We present a high precision 3D optical tweezers setup, which is incorporated in an optical inverted microscope and uses the detection of backscattered light to measure forces in the sub-pN regime and to manipulate single DNA molecules. With this novel setup, single dsDNA-molecules were threaded into a solid-state nanopore and the electrostatic forces and the ionic currents through the pore were measured simultaneously. In the force-distance diagrams, individual force steps could be observed for each DNA-molecule entering the nanopore and distinct force signals could be identified upon actively withdrawing the single DNA-molecule out of the nanopore. We found that binding of dedicated protein ligands (peroxiredoxin, E. coli RNA-polymerase, and RecA) to dsDNA caused a significant change in the apparent electrostatic forces that are required to thread and unthread the DNA-ligand-complex through the nanopore. Furthermore, we were able to detect the exact position of the binding ligand along the DNA strand with nanometer precision.

BP 6.7 Mon 17:15 Poster B1

Visualisation of PCNA monoubiquitination in vivo by single pass spectral imaging FRET microscopy — •Christopher Batters[1], Hannah Zhu[2], and Julian Sale[2] — [1]Institute of Physiology, Ludwig-Maximilians-Universität, Pettenkofestr. 12, 80336 München, Germany — [2]Medical Research Council Laboratory of

Molecular Biology, Division of Protein & Nucleic Acid Chemistry, Hills Road, Cambridge, CB2 0QH, U.K.

Monoubiquitination of the DNA sliding clamp, PCNA, plays a central role in the control of damage bypass during replication. By combining a widely-spaced FRET donor/acceptor pair (CFP and mRFP) with spectral imaging, we have developed a simple method for the visualisation of PCNA monoubiquitination in both fixed and live cells with a single imaging pass. We validate the method with genetic controls in the avian cell line DT40 and examine the intracellular dynamics of PCNA ubiquitination following subnuclear UV irradiation. This general approach is likely to be of utility for live imaging of monoubiquitination and sumoylation of a wide range of substrates in vivo.

BP 6.8 Mon 17:15 Poster B1

Stretching of DNA/TmHU-protein complexes in SMD simulations — •Carsten Olbrich and Ulrich Kleinekathöfer — Jacobs University Bremen, Campus Ring 1, 28759 Bremen, Germany

The protein HU is a member of a family of prokaryotic proteins that interacts with the DNA in a non-specific way [1]. Its major function is the binding, compaction and stabilization of DNA. Steered molecular dynamic (SMD) simulations are applied to DNA which is bound to the HU protein of the bacteria Thermotoga maritima (TmHU). Using these all-atom simulations including explicit water and about 80,000 atoms in total, we are able to gain insight into the discrete disruptions events which occur when the DNA releases from the protein body. These disruptions were first observed in experiments performed with optical tweezers [2]. We will present a detailed view of those events on the atomistic scale.

[1] R. Dame and N. Goosen, FEBS Lett. **529**, 151 (2006).
[2] M. Salomo, F. Kremer et al., J. Mol. Biol. **359**, 769 (2006).

BP 6.9 Mon 17:15 Poster B1

Unfolding mechanisms and the free energy landscape of the DNA i-motif — •Jens Smiatek and Andreas Heuer — Westfälische Wilhelms-Universität Münster, Institut für Physikalische Chemie, 48149 Münster, Germany

Since the discovery of the DNA i-motif, the formation and function of this specific structure has attracted broad interest. Even the pH-dependent reversible folding/unfolding mechanism has been nowadays used in technological applications like in the construction of nanocontainers. The unfolding mechanism has been investigated in high temperature simulations and is characterized in terms of the eigenvectors. Furthermore we present the results of Molecular Dynamics simulations for the free energy landscape which has been computed by a recently developed method for several collective variables.

BP 6.10 Mon 17:15 Poster B1

Towards sub second imaging of DNA compaction by AFM — •Jan Knappe[1], Sebastian Hanke[1], Szabolcs Sörös[2], Christoph F. Schmidt[1], Wolfgang Fischle[2], and Iwan A. T. Schaap[1] — [1]III. Physkalisches Institut, Georg-August-Universität, 37077 Göttingen — [2]Max Planck Institute for Biophysical Chemistry, 37077 Göttingen

To compact and organize DNA the eukaryotic cell has developed several packaging steps. DNA is wrapped around histones to form nucleosomes, which in turn can be linked by other proteins to form larger aggregates. To study this system on a single molecule level we have set out to use atomic force microscopy (AFM) in liquid to image the binding and unbinding of DNA to the histone octamer. However to be able to study the dynamics of nucleosomal DNA on a biological relevant time scale, we need techniques that permit image acquisition in a second or faster. We will present our first results that combine imaging at low forces (~100 pN) with a high temporal resolution.

BP 6.11 Mon 17:15 Poster B1

Bayesian inference based evaluation of DNA hairpin dynamics — •Wolfgang Kügel, Adam Muschielok, and Jens Michaelis — Ludwig-Maximilians-Universität, Munich, Germany

Fluorescence-correlation-spectroscopy (FCS) combined with FRET is a powerful tool to analyze dynamics in biological systems. In comparison to other approaches this technique is not limited to a narrow range of rates and can detect dynamics from the ns to s time-scale. However, the key problem is to extract the rates hidden in the correlation curve by fitting a set of parameters. Several different fitting approaches have been described in recent years but the extraction of relevant information is still limited by the fact that the set of starting values chosen predefines the result. This happens as reasonably different sets of parameters result in fitting curves that describe the data equally well. To evaluate and weigh all possible fit results for our data we have used a Bayesian inference approach and globally evaluated all information available. A first application of this approach is shown based on a representative selection of different FRET pairs bound to a hairpin DNA. We discuss how dye selection can influence the rates of hairpin opening and closing.