BP 13: Posters: DNA/RNA and related enzymes

Time: Tuesday 9:30–12:30 Location: P1

BP 13.1 Tue 9:30 P1

Local melting of ds-DNA within ultrathin amphiphilic films — •Caroline Falk, Hua Liang, Nikolai Severin, and Jürgen P. Rabe — Department of Physics Humboldt-Universität zu Berlin

DNA-replication is an important process in the human body. Replication of double-stranded (ds)-DNA requires its local unwinding, i.e. melting into two single strands (1). 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. Preliminary work has demonstrated that this transition can be investigated on a graphite surface, pre-coated with an amphiphilic layer imaged with scanning force microscopy (3). ds-DNA can be stretched by an amphiphilic layer. This leads to a local splitting of the ds-DNA into two single strands and overwinding of the rest of the vector ds-DNA. This effect can be analyzed as a function of plasmid length and base sequences. We demonstrate here our efforts to identify the location of the local melting within the ds-DNA by marking specific locations within the DNA.

(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, W.Zhuang, N.Severin, J.P.Rabe Control of Single DNA Conformation on a Nanostructured Template (to be published).

BP 13.2 Tue 9:30 P1

Quantitative DNA Overstretching Using Optical Tweezers — Roland Hillmann, André Spiering, Andy Sischka, and •Dario Anselmetti — Experimental Biophysics, Bielefeld University, 33615 Bielefeld, Germany

We investigated the binding of histonecomplexes to DNA by monitoring the dynamic structure of individual nucleosomes by optical tweezers with single molecule force spectroscopy. 16.4um long biotinylated dsDNA is attached to two streptavidin coated microspheres, one held by a micropipette, as well as the other by the optical trap, respectively. The histones in our experiment contain all of the natural proteins (H2a, H2b, H3, H4) including the linker histone-like protein (H1) to form higher order structures. We observed distinct sawtooth patterns, that can be interpreted as the release of individual nucleosome complexes and will discuss the observed phenomena in the framework of histone complex formation.

BP 13.3 Tue 9:30 P1

Nucleosome Breathing Facilitates Rapid DNA Packaging Into Chromatin — •Brendan Osberg, Johannes Nuebler, and Ulrich Gerland — Arnold-Sommerfeld Center for Theoretical Physics and Center for NanoScience, Theresienstraße 37, 80333 München

In eukaryotic cells DNA is packaged into chromatin: Nucleosomes, each consisting of 147 base pairs of DNA wrapped around a histone protein octamer, cover approximately 90% of the DNA. However, the mechanisms whereby cells achieve such a high coverage within a short time, e.g. after DNA replication, remain poorly understood.

It is well known that random sequential absorption processes of hard particles on a line lead to extremely slow filling kinetics beyond about 75% coverage, due to a "jamming" behavior. Of course, ATP consuming "remodeler" enzymes can speed up the filling of the DNA with nucleosomes.

However, we show that the breathing property of nucleosomes, where DNA transiently partially unwraps from the histones due to thermal fluctuations, can already eliminate the jamming behavior and alleviate the kinetic problem. We also consider effects whereby the steady progression of the replication fork could enable fast filling in its wake.

BP 13.4 Tue 9:30 P1

Measuring DNA translocation through nanopores in graphene and carbon nanomenbranes with Optical Tweezers — •Sebastian Knust, Andre Spiering, Andy Sischka, and Dario Anselmetti — Experimental Biophysics & Applied Nanoscience, Faculty of Physics, Bielefeld University, 33615 Bielefeld, Germany

The forces acting on DNA during translocation through a nanopore were measured with Optical Tweezers. We developed a video-based force detection and analysis system allowing for virtually interference-free axial force measurements with sub-piconewton precision [1].

We previously measured the translocation of dsDNA through 20 nm thick Si2N3 membranes (0.1 pN/mV for pores $\geq$ 30 nm) [2, 3]. Carbon nanomembranes and graphene with a thickness of 3 nm and 0.3 nm respectively allow for even more sensitive measurements.

We show the controlled translocation of a single dsDNA strand attached to a microbead with an overall force resolution of $\pm$0.5 pN at a sample rate of 123 Hz.

[1] S. Knust et. al., Rev. Sci. Instrum. **83**, 103704 (2012)

[2] A. Spiering et. al., Nano Lett. **11**, 2978 (2011)

[3] A. Sischka et. al., in preparation

BP 13.5 Tue 9:30 P1

Single Molecule Localization Microscopy of Chromatin Structures — •Udo Birk[1,2], Kirti Prakash[1], Aleksander Szczurek[1], Hyun-Keun Lee[1], and Christoph Cremer[1,2] — [1]Institute of Molecular Biology (IMB), Ackermannweg 4, 55118 Mainz, Germany — [2]Kirchhoff Institute for Physics, Heidelberg University, 69120 Heidelberg, Germany

Understanding the structural and organizational aspects of chromatin at different stages of cell differentiation as well as at various phases of the cell cycle is one of the many promising applications of super-resolution microscopy. It is challenging to study e.g. the organization of the different proteins which form the basis of chromosomal superstructures, due to limitations of conventional Light Microscopy (LM) and of Electron Microscopy (EM). To this end, structured illumination microscopy (with an optical resolution of about 100 nm in the object plane) could provide an improved resolution of intact cell nuclei and of the chromatin therein.

We report results on visible light based single molecule localization microscopy (SMLM) of chromatin structures in intact cell nuclei, and studied these structures at different stages of the cell cycle using the SMLM method of Spectral Position Determination Microscopy (SPDM). We analyzed the distribution of DNA and of associated proteins. The images obtained show a dramatic increase in light optical resolution of chromatin structures. Novel labeling techniques are required to make full use of SMLM visualization of DNA also directly labeled, i.e. without fluorescence in-situ hybridization.

BP 13.6 Tue 9:30 P1

Effects of molecular crowding on promoter finding — •David Gomez and Stefan Klumpp — Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

All biological functions of DNA depend on the recognition of specific sequences by site-specific DNA-binding proteins. Binding of these proteins to their binding sites is believed to occur through a facilitated diffusion process that combines one-dimensional diffusion along the DNA with three-dimensional diffusion in the bulk solution.

This model has been assumed to be general for all DNA-binding proteins even though the conditions, such as protein concentrations, are different for different DNA-binding proteins. The objective of this work is to model the dynamics of the RNAP-promoter binding under different concentrations of RNAPs and other proteins that act as 'obstacles.' To do that, we place a linear DNA template with its promoter, RNAPs and obstacles on a 3D lattice, and determine the RNAP-promoter binding rates at different conditions. Our results suggest that an acceleration in the binding process occurs only at some obstacle concentrations and non-specific binding times of the RNAP on the DNA.

BP 13.7 Tue 9:30 P1

Dielectric spectroscopy of DNA up to 110 GHz — Elena Ermilova, Frank Bier, and •Ralph Hölzel — Fraunhofer Institute for Biomedical Engineering Am Mühlenberg 13, 14476 Potsdam-Golm, Germany

Dielectric studies of DNA aqueous solutions still contribute to a better understanding of the interaction mechanisms between biological molecules, solvent, their ionic environment and electromagnetic fields at upper GHz and lower THz frequencies. Reflection measurements using a vector network analyzer allow a fast and uncomplicated determination of permittivity spectra from the measured complex reflection

coefficient. In the present study we report the experimental results on dielectric relaxation of Na-DNA at various concentrations. Permittivity measurements were performed in the broad frequency range between 25 MHz and 110 GHz in a single sweep by means of a vector network analyzer. The permittivity spectra of DNA solutions exhibit a complex behaviour with several dispersion regions attributed to different relaxation mechanisms. Resulting from fitting to the double Cole-Cole model we resolved two dispersion regions: around 100 MHz and 20 GHz, respectively, and analysed their relaxation strengths and relaxation times.The wide frequency range provides possibilities for better analysis of the relaxation process attributed to the water dipoles in the presence of DNA macromolecules. The small dimension of the coaxial sensor allows to reduce the sample volume, which opens new possibilities for dielectric spectroscopy of well defined highly concentrated DNA solutions, as well as biological and synthetic polymer solutions.

BP 13.8 Tue 9:30 P1

Experiments of DNA-Ligand-Complexes with Optical and Magnetic Tweezers — •Ying Wang[1], Susan Haji Samo[1], Andy Sischka[1], Helene Schellenberg[1], Katja Tönsing[1], Thomas Jany[2], Thorsten Glaser[2], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanoscience, Bielefeld University, 33615 Bielefeld, Germany — [2]Lehrstuhl für Anorganische Chemie I, Bielefeld University, 33615 Bielefeld, Germany

We investigated the specific binding of Co-, Cu- and Ni-dinuclear metal complexes (DNMCs) to dsDNA with optical (OT) and magnetic tweezers (MT). DNMCs bind to the DNA backbone, hydrolyse phosphate esters (cutting functionality) and thus prevent the particular DNA segment from being replicated or transcribed. In the OT stretching experiments, we have observed force peaks, suggesting knot or coil formation of DNA entanglements, since the aromatic groups of neighbouring DNMCs can interact with each other via π-stacking. Detecting these complexations have proved the binding of DNMCs to DNA. In twisting experiments with MT, we have evidenced that 200μM Cu-DNMC shows cutting functionality. Because of the created nicks, the DNA molecule can no further be twisted. Combining with molecular recognition techniques DNMCs could be utilized on medical applications as a possible drug dealing with cancers in the future.