BP 24: Posters: DNA/RNA and Related Enzymes

Time: Thursday 17:30–19:30 Location: Poster A

BP 24.1 Thu 17:30 Poster A

Information transfer and readout in complex DNA mixtures — Harish Bokkasam[1] and •Christian Trapp[2] — [1]Institute for Biological Experimental Physics, University of Saarland, Saarbruecken, Germany — [2]Institute for Biological Experimental Physics, University of Saarland, Saarbruecken, Germany

Project: Development of an enzyme based method for the copy of oligos with predetermined length from biological template, given knowledge of the therein contained oligonucleotide sequence. In this project, we modify the conventional PCR technique by to generate linearly amplified copies of single stranded oligos. To recover the ssDNA transcripts from the linear PCR reaction, various techniques like gel extraction, column filtration, magnetic beads are used. These purified transcripts are fluorescently labelled in different ways like in-strand labelling and 5/3 end labelling for microarray analysis. To visualize ssDNA fragments of very short length and low concentration here denatured polyacrylamide gel electrophoresis with silver staining is used. From this gel electrophoreses we can approximate the length of the product.

Conclusion: Our method has given very promising results so far. An improvement would be to narrow the length distribution of the transcribed sequences further. Currently we are validating our technique by using a DNA microarray.

BP 24.2 Thu 17:30 Poster A

Stable conformations of a single stranded deprotonated DNA i-motif — •Jens Smiatek[1], Dongsheng Liu[2], and Andreas Heuer[1] — [1]Institut für Physikalische Chemie, WWU Münster, D-48149 Münster, Germany — [2]Department of Chemistry, Tsinghua University, Beijing 100190, P. R. China

We present Molecular Dynamics simulations of a single stranded deprotonated DNA i-motif in explicit solvent. Our results indicate that hairpin structures are stable equilibrium conformations at 300 K. The entropic preference of these configurations is explained by strong water ordering effects due to the present number of hydrogen bonds. We observe a full unfolding at higher temperatures in good agreement to experimental results.

BP 24.3 Thu 17:30 Poster A

Models of Base Excision Repair — •Laurin Lengert — TU Darmstadt, Hessen

Our goal is to study simple models of DNA repair networks. One such network is the base excision repair network which is involved in the repair of single strand breaks. We will discuss the minimal set of repair proteins needed for a model that is able to reproduce the time course of protein recruitment data revealed by experiments. Furthermore, we will analyze how the properties of these simple models affect the data produced in computer simulations. This in turn enables us to interpret various features of the experimental curves in terms of the underlying processes.

BP 24.4 Thu 17:30 Poster A

DNA in an infinite nanochannel — •Wojciech Müller, Stefan Kesselheim, and Christian Holm — ICP, Stuttgart, Deutschland

We perform full atomistic molecular dynamics simulations, where an infinite b-DNA helix is enclosed in an infinite channel. The DNA is solvated in an explicit SPC water model, enriched with ions of various concentrations. The ion distribution and the flow is then compared to results from coarse grained models. The goal is to find the limits of these coarse grained models and identify effects which are not captured by them.

BP 24.5 Thu 17:30 Poster A

mechanical properties of DNA through numerical method and monte carlo simulation — •hassan chatrsahar[1] and mohammad hossein yamani[2] — [1]Islamic Azad University of Takestan, Takestan, Iran — [2]Johannes Gutenberg University, Mainz, Germany

DNA as an anisotropic flexible polymer contains bioinformation of all living cells. Interactions between DNA and proteins that cause deformations in the structure of DNA are essentially ubiquitous during many life processes inside cells. We study the persistence length of DNA in two and three dimensions, considering the elastic bending anisotropy and twist-bend coupling through Worm-Like Chain model (WLC) and Metropolis Monte Carlo simulation. We compare the persistence length of DNA in two and three dimensions. We show although twist-bend coupling does not affect the persistence length in three dimensions, it increases the persistence length in two dimensions by a few percents. Also, through Elastic Rod model we derive the energy equation of a deformed DNA and by minimizing the conformation energy and solving the equations through numerical method, the spatial conformation of a DNA under various boundary conditions and integral constrains is studied. After that, effects of the twist-bend coupling and anisotropy in DNA conformation and its energy are probed.

BP 24.6 Thu 17:30 Poster A

Ab Initio Study of The Electric Hyperfine Interactions: DNA Bases With Metal Cations (Cd and In). — •Philippe Alexandre Divina Petersen, Marcos Brown Gonçalves, and Helena Maria Petrilli — Instituto de Física, DFMT, Universidade de São Paulo, São Paulo, SP, Brazil

The hyperfine interactions provide information around the probe atom. This information is given in nanometer scale and through interactions between the nuclear quadrupole moment of the atom and the charges in its surroundings. Nuclear quadrupole coupling constants (vQ) in molecules depends on the nuclear quadrupole moment and the Electric Field Gradient (EFG) at the nucleus. Thus, the theoretical analysis of EFG is interesting because it can help both interpreting experimental results and estimate the adequacy of structural models. Metal cations bound to DNA bases can change many aspects of the base pairing [1]. They also facilitate or difficult the breakdown of this bases, depending on the location where the Cd and In are linked to DNA. The methodology used for the electronic structure calculations is based on the Kohn-Sham [2] scheme of the Density-Functional Theory (DFT). The computational code used is the Projector Augmented-Wave Method [3] combined with the Car-Parrinello [4] scheme(CP-PAW).

[1] J. V. Burda, J. Sponer, J. Leszczynski, P. Hobza,J.Phys. Chem.B., 101, 9670 (1997). [2] W. Kohn, L. J. Sham, Phys. Rev. B., 140,1133 (1965). [3] P. E. Blöchl, Phys. Rev. B., 50, 17953 (1994). [4] R. Car e M. Parrinello, Phys. Rev. Lett. 55, 2493 (1985). [5] A. S. Silva,A. W. Carbonari (Private Communication).

BP 24.7 Thu 17:30 Poster A

Imaging of DNA overwinding and splitting — •Hua Liang, Nikolai Severin, and Jürgen Rabe — Department of Physics, Humboldt University Berlin, Newtonstr. 15, D-12489 Berlin, Germany

Unwinding and melting a DNA double helical structure at a specific region is the initiation step for DNA replication, the precise mechanism of which remaining still ambiguous (1). Experimental and theoretical studies on stretching and twisting of double-stranded (ds-) DNA reveal its chirality by mechanical twist-stretch coupling: small stretching along the DNA backbone induced torsional stress along the molecular backbone and vice versa (2). It has been theoretically shown that ds-DNA may release its torsional stress inhomogeneously along the backbone with localized, sequence-dependent structural failure to preserve its B-form, when supercoiling is not allowed (3). However, the pulling experiments were carried out in solution, where it is difficult to access the direct conformational changes during stretching. Here we report the experimental observation of plasmid DNA (pUC19 and pBR 322) overwinding with local splitting of the double helix into two single strands when stretched on a surface. Only one split is observed for different lengths of DNA, with the splitting length proportional to the total length. We discuss a possible unwinding and splitting mechanism analogue to many biological processes which involve DNA in vivo such as replication, transcription initiation, and DNA repair. [1]M. L. Mott, J. M. Berger, Nature Reviews Microbiology 5, 343 (2007. [2]J. Gore et al., Nature 442, 836 (2006). [3]G. L. Randall, L. Zechiedrich, B. M. Pettitt, Nucleic Acids Research 37, 5568 (2009).

BP 24.8 Thu 17:30 Poster A

Thermal and photothermal dissociation of DNA-gold nanoparticle networks of different sizes — •Malte Linn[1], Anne Buchkremer[2], Ulrich Simon[2], and Gero von Plessen[1] — [1]Inst. of Physics (IA), RWTH Aachen University, Germany — [2]Inst. of Inorganic Chemistry, RWTH Aachen University, Germany

The functionalization of gold nanoparticles (AuNPs) with DNA allows the construction of DNA-AuNP networks, which have the ability to act as both optical sensors and actuators. By using optical extinction spectroscopy (OES), it is possible to monitor the state of the networks, since the mutual electromagnetic coupling of individual particles inside each network leads to a red shift of the plasmon peak position with respect to single particles. Since DNA dehybridization is temperature-sensitive, network dissociation can be triggered either by mere conventional heating or by introducing additional photothermal heating via irradiation with cw laser light. In this work, we tailor the size of the DNA-AuNP networks by varying the ratio of complementarily functionalized AuNPs. The size dependence of the optical and chemical properties of the networks is investigated and quantified by means of OES and dynamic light scattering. These properties depend on the network size, because larger networks involve a greater number of AuNPs and DNA bonds. The influence of additional photothermal heating on the dissociation temperature associated with each network size is also investigated.

BP 24.9 Thu 17:30 Poster A

Kinetics of DNA hairpin-loops in crowded and non-crowded fluids — •Olivia Stiehl, Maria Hanulova, Gernot Guigas, and Matthias Weiss — University of Bayreuth, Bayreuth, Germany

Single-stranded DNA hairpin-loops are involved in many biological processes, e.g. in the regulation of gene expression and DNA recombination. Investigating the kinetics of hairpin loops yields a better quantitative understanding of such processes and therefore may help to improve, for instance, the efficiency of antisense drugs. Using a combination of fluorescence correlation spectroscopy and fluorescence energy transfer, we have investigated the kinetics of thermally induced DNA hairpin-loop fluctuations. In particular, we were interested in the influence of macromolecular crowding on the time constant of opening/closing of the DNA loop and on the fraction of open DNA loops. Our measurements were performed with the crowding agent dextran at different concentrations and molecular weights. We found that both, a purely viscous as well as a crowded environment lead to a slower kinetics. Furthermore, our measurements indicate that a viscous surrounding does not affect the fraction of open DNA loops, whereas an increased crowding enhanced the fraction of closed DNA loops.

BP 24.10 Thu 17:30 Poster A

AFM imaging of conformational changes in DNA after hydroxyl radical attack — Janine Wilken[1] and •Stephan Block[2] — [1]Institut für Physik, Ernst-Moritz-Arndt Universität, Felix-Hausdorff-Str. 6, D-17487 Greifswald, Germany — [2]ZIK HIKE - Zentrum für Innovationskompetenz Humorale Immunreaktionen bei kardiovaskulären Erkrankungen, Fleischmannstr. 42 - 44, D-17487 Greifswald, Germany

AFM is used to directly visualise changes in the conformation of DNA after attack of reactive oxygen species (ROS). In detail, the plasmid pBR322 is mixed with Fentons reagent, whereby the ratio R of hydroxyl radical molecules to DNA base pairs is varied. After the radical attack the negatively charged plasmid is immobilized onto mica surfaces that are functionalized with positively charged poly(allylamine hydrochloride) (PAH). At R = 2.5 most of the plasmid adopts the supercoiled (double-stranded) conformation and only few chains are split up by the radical attack. An increase in hydroxyl radical concentration also increases the power of the radical attack, leading to a rising number of shorter (single-stranded) fragments of plasmids. Interestingly, these fragments are often surrounded by plateaus (0.2 nm in height) which might be attributed to the aggregation of base pairs, which are chemically modified after the radical attack.

BP 24.11 Thu 17:30 Poster A

Optical Tweezers Force Spectroscopy on Protein Nanopores in Lipid Bilayers — •Andy Sischka[1], Lorena Redondo-Morata[2], Helene Schellenberg[1], Andre Spiering[1], Sebastian Knust[1], Katja Tönsing[1], Fausto Sanz[2], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanosciences, Faculty of Physics, Bielefeld University — [2]Physical Chemistry Department, University of Barcelona

We are aiming to investigate the controlled translocation of individual DNA molecules through protein nanopores with our quantitative 3D optical tweezes system, similarly to recent single molecule threading experiments through solid-state nanopores (nanopore force spectroscopy) [1, 2]. There, quantitative translocation forces and ionic current signals of individual DNA molecules and DNA-protein complexes were identified and related to the respective protein type and charge.

Since we want to improve our understanding of the molecular translocation dynamics and increase the sensitivity of the translocation force and the ionic current, we extend these experiments towards biological nanopores and membranes by measuring both current and forces acting on a single-stranded DNA molecule that will be threaded through a protein nanopore inside a lipid bilayer. This bilayer is immobilized on a Si_3N_4 membrane support by vesicle spreading or Langmuir-Blodgett transfer. We will discuss first results with single-pore forming proteins like alpha-hemolysin.

[1] A. Sischka et al.: J. Phys.: Condens. Matt. **22**: 454121 (2010)
[2] A. Spiering et al.: Nano Letters **11**: 2978 (2011)

BP 24.12 Thu 17:30 Poster A

Video-based axial force analysis for 3D quantitative optical tweezers — •Sebastian Knust, Andy Sischka, Andre Spiering, and Dario Anselmetti — Experimental Biophysics and Applied Nanoscience, Faculty of Physics, Bielefeld Institute for Biophysics and NanoScience (BINAS), Bielefeld University, 33615 Bielefeld, Germany

We developed and included video-based axial force analysis into our previously described optical tweezers setup [1]. By measuring the radius of a trapped microbead we achieve an overall force resolution along the z-axis in the range of 0.2pN with a bandwidth of 120Hz, only limited by our CCD camera. With this video-based method we overcome the remaining weak interference effects in backscattered light based force analysis when operating a microsphere in the vicinity of an interface.

We tested our setup by investigating the controlled threading and translocation of individual lambda-DNA molecules with and without attached DNA-binding ligands through solid-state nanopores and comparing these results with previous measurements realized with photodiode intensity detection [2, 3].

[1] A. Sischka et. al., Rev. Sci. Instrum. **79**, 063702 (2008)
[2] A. Sischka et. al., J. Phys.: Condens. Matter **22**, 454121 (2010)
[3] A. Spiering et. al., Nano Lett. **11**, 2978 (2011)

BP 24.13 Thu 17:30 Poster A

Nanopore Translocation Dynamics of a Single DNA-Bound Protein — •Andre Spiering[1], Sebastian Getfert[2], Andy Sischka[1], Katja Tönsing[1], Karsten Rott[3], Peter Reimann[2], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanoscience, Bielefeld University, 33615 Bielefeld, Germany — [2]Soft Matter Theory, Bielefeld University — [3]Thin Films and Physics of Nanostructures, Bielefeld University

The single molecule translocation dynamics of various dsDNA-protein complexes upon threading through a solid-state NP was investigated by quantitative 3D-optical tweezers (OT) in the presence of an electric field (NP force spectroscopy). In our single molecule translocation experiments, we find distinct asymmetric force signals that depend on the protein type and charge, the DNA elasticity and its counterionic screening in the buffer **[1,2]**. In order to increase the resolution of these force signals even further we drastically decreased the thickness of the membrane containing the nanopore. We prepared graphene monolayers by exfoliation from graphite, transferred them to Si/Si_3N_4-support chips and milled the freestanding graphene membrane (with a focused ion beam or a TEM) to produce graphene nanopores of diverse size and shape. Comparing the results for different membranes and nanopores is the basis to further understand the exact physical properties of such translocation dynamics and may lead to the development of novel nanopore sensing and sequencing devices.

[1] A. Sischka et al.: *J. Phys.: Condens. Matter* **22**: 454121 (2010)
[2] A. Spiering et al.; *Nano Letters* **11**: 2978-2982 (2011)