BP 13: DNA/RNA and Related Enzymes

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

Topical Talk BP 13.1 Wed 9:30 H 1058

Chemo-mechanics of a ring-shaped helicase during unwinding — •Michael Schlierf[1,2], Ganggang Wang[3], Xiaojiang Chen[3], and Taekjip Ha[1,4] — [1]University of Illinois at Urbana-Champaign, Physics Department and Center for Physics of Living Cells, Urbana, Illinois — [2]B CUBE - Center for Molecular Bioengineering, TU Dresden — [3]University of Southern California, Department of Biological Sciences — [4]Howard Hughes Medical Institute, Urbana, Illinois

Most replicative helicases are hexameric ring-shaped enzymes and are essential for cell survival. Despite extensive biochemical, structural and single-molecule investigations, how the translocation activities are utilized in the mechanical process of dsDNA unwinding are poorly understood. We investigated DnaB-family helicase G40P using a single molecule fluorescence-based unwinding assay with a single base pair resolution. The high-resolution assay revealed that G40P is an ultraweak helicase that stalls at barriers as small as a single GC base pair and is a motor that moves with the step size of a single base pair. We directly observed the long-postulated activity of helicase slippage that is markedly enhanced under conditions that slow forward progression, but is fully suppressed by the primase DnaG.

BP 13.2 Wed 10:00 H 1058

The Speed of Ribosomes — •Sophia Rudorf, Angelo Valleriani, and Reinhard Lipowsky — Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Am Mühlenberg 1, 14476 Potsdam

To synthesize a protein a ribosome moves along the codons of a messenger RNA (mRNA) and takes up corresponding aminoacylated transfer RNAs (aa-tRNAs). During this process called translation elongation the ribosome does not always proceed at the same speed. Here we present an analytical model to calculate codon-specific elongation rates based on aa-tRNA concentrations and codon usages. The model takes into account non-cognate aa-tRNAs that compete with cognate aa-tRNAs as well as the number of translating ribosomes. Using available in vivo data and codon usages obtained from mRNA Deep Sequencing we computed the elongation rates for Escherichia coli. Results of stochastic simulations based on these elongation rates coincide well with experimental data. We found that increasing the number of translating ribosomes strongly decreases the availability of frequently used aa-tRNAs. This leads to comparably low elongation rates of some abundant codons, in contrast to the generally high correlation of elongation rates and codon usages.

BP 13.3 Wed 10:15 H 1058

Nematic ordering due to intrinsic chain stiffness causes DNA molecules packed in phage capsids to preferentially form torus knots — Daniel Reith[1], Andrzej Stasiak[2], Peter Cifra[3], and •Peter Virnau[1] — [1]Department of Physics, Uni Mainz — [2]Center for Integrative Genomics, UNIL, Lausanne, Switzerland — [3]Polymer Institute, Slovak Academy of Sciences, Bratislava, Slovakia

When mature bacteriophages such as P2 or P4 are assembled in infected cells, a long linear DNA molecule is loaded into the phage capsid and arranges itself in a toroidal, nematic phase. Intriguingly, experiments show that the DNA is not only highly knotted, but also exhibits a rather uncommon knot spectrum. Observation that DNA molecules in bacteriophage capsids preferentially form torus knots provide a sensitive gauge to evaluate various models of DNA arrangement in phage heads. We demonstrate with computer simulations of a simple bead-spring model that an increasing chain stiffness not only leads to nematic ordering and a (somewhat counter-intuitive) increase of knottedness, it is also the decisive factor in promoting formation of DNA torus knots in phage capsids.

BP 13.4 Wed 10:30 H 1058

The binding of monoclonal antibodies and tau-peptides - how two binding sites add up to form a stable specific bond — •Carolin Wagner, David Singer, Tim Stangner, Christof Gutsche, Olaf Ueberschär, Ralf Hoffmann, and Friedrich Kremer — Leipzig University, Leipzig, Germany

Optical tweezers-assisted dynamic force spectroscopy (DFS) is employed to investigate specific receptor/ligand interactions on the level of single binding events [1]. Here, the specific binding of the anti-human tau monoclonal antibody (mAb), HPT-101, to synthetic tau-peptides is analyzed. Amongst others, the massive accumulation of tangles that mainly consist of hyperphosphorylated tau-proteins is characteristic for Alzheimer*s disease. The sorts of tau-peptides, which are used in this study, contain either one phosphorylation, at Thr231 and Ser235, respectively, or they are phosphorylated at both sites. From measurements using ELISA it is known, that the HPT-101 binds only specifically to the double-phosphorylated tau-peptide. The results obtained by DFS show, that HPT-101 binds also to each sort of the mono-phosphorylated peptides. By analyzing the measured rupture-force distributions characteristic parameters like the lifetime of the bond without force t0, the characteristic length xts and the free energy of activation Delta G are determined for all interactions. Thereby it can be shown how the attachments of HPT-101 with the mono-phosphorylated peptides add up in the case of the double-phosphorylated peptide in order to form the strong specific binding.

[1] C. Wagner et al., Soft Matter, 2011, 7 (9), 4370 - 4378

BP 13.5 Wed 10:45 H 1058

Electrophortic mobility of DNA-grafted single colloids as studied by optical tweezers — •Ilya Semenov, Christof Gutsche, Mahdy M. Elmahdy, Olaf Ueberschär, and Friedrich Kremer — Institute for Experimental Physics I, University of Leipzig, Linnéstrasse 5, 04103 Leipzig, Germany

The electrophoretic mobility of single particles grafted with double stranded (ds) DNA is studied by use of optical tweezers (OT) accomplished with fast position detection (Single Colloid Electrophoresis [1, 2]). Parameters to be varied are the concentration (0.01 mMol/l - 1 Mol/l) and valency (KCl, $CaCl_2$, $LaCl_3$) of the ions in the surrounding aqueous medium, but as well the contour length (250, 1000 and 4000 base pairs) of the grafted chains. For the DNA-grafted colloids a pronounced decrease of the electrophoretic mobility is observed in comparison to blank particles under identical conditions. The findings are discussed in terms of the Standard Electrokinetic Model [3]. The electrophoretic mobility of a ds-DNA-grafted single colloid at high ionic strength can be understood quantitatively within the limits of the linearized Poisson-Boltzmann equation.

[1] I. Semenov et al., Journal of Physics: Condensed Matter 22, 494109 (2010).

[2] I. Semenov et al., Journal of Colloid and Interface Science 337, 260 (2009).

[3] R. W. O'Brien, and L. R. White, JCS, Farad. Trans. 2 74, 1607 (1978).

BP 13.6 Wed 11:00 H 1058

RNA folding dynamics studied with a structure-based model — •Michael Faber and Stefan Klumpp — Max Planck Institute of Colloids and Interfaces Potsdam

RNA molecules form three-dimensional structures as complementary bases form bonds and the molecule coils. These structures determine the function and biochemical activity of the molecule. For example, the presence or absence of a specific RNA structure can invoke transcriptional pauses or terminate the transcription altogether. We have developed a structure-based model for studying the folding dynamics of RNA secondary structures. To simulate the dynamics, we use a Monte-Carlo method with Metropolis rates, where the basic steps are the closing or opening of one native contact. We apply this model to the folding and unfolding of simple RNA structures in the presence and absence of an external force.

15 min break

BP 13.7 Wed 11:30 H 1058

Computer simulation of chromatin: Effects of nucleosome positioning on chromatin structure — •Oliver Müller[1], Robert Schöpflin[1], Nick Kepper[2], Ramona Ettig[2], Karsten Rippe[2], and Gero Wedemann[1] — [1]CC Bioinformatics, University of Applied Sciences Stralsund, Zur Schwedenschanze 15, 18435 Stralsund, Germany — [2]Deutsches Krebsforschungszentrum & BioQuant, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany

The three-dimensional structure of chromatin is a key factor for DNA accessibility, replication and repair. Most theoretical models of chromatin imply a static, periodical positioning and uniform occupancy of

nucleosomes. However, recent studies suggest a dynamic nucleosome positioning, which is both actively regulated by chromatin-remodeling complexes and passively influenced by thermal fluctuations. In turn, nucleosomes deviating from regular positions can introduce changes into chromatin fiber structure. To investigate the effects of nucleosome repositioning, we carried out Monte Carlo simulations with a coarse-grained chromatin model incorporating elastic fiber properties as well as electrostatic and internucleosomal interactions. We created fiber models based on experimental results and modified these by repositioning nucleosomes by a range of base pairs. After simulation, the chromatin energy landscape and fiber shape were analyzed. We observed a significant energy barrier against nucleosome repositioning which is larger than thermal fluctuations but within the range of ATP-dependent biological processes. Moreover, the region proximate to a repositioned nucleosome revealed an increased kinking susceptibility.

BP 13.8 Wed 11:45 H 1058

Confinement Driven Spatial Organization of Semiflexible Ring Polymers — Miriam Fritsche and •Dieter Heermann — Universität Heidelberg, Institut für Theoretische Physik, Philosophenweg 19, D-69120 Heidelberg

We investigate conformational properties of a semiflexible ring polymer in confined spaces. Taking into account the competing interplay between configurational entropy, bending energy and excluded volume, we elucidate the role that different geometrical constraints can play in shaping the spatial organization of biopolymers. While elongated, rod-like geometries reduce the amount of chain overcrossings and induce a pronounced ordering with respect to the long axis of the surrounding envelop, there exists no preferred orientational axis in case of spherical confinement. Upon increasing the system density and rigidity of the chain, the polymer migrates from the center of the accessible space towards the surrounding surface forming a spool-like structure known for DNA condensation within viral capsids. The existence of distinguished loop sizes for different confining geometries might influence co-localization in biopolymers necessary for the genome-wide coordination of gene expressed. Thus, the advantages of certain geometric constraints such as spherical confinement of viral DNA in a capsid or the rod-shaped envelop of the circular chromosome in Escherichia coli could be one driving force for controlling proper biological functioning.

BP 13.9 Wed 12:00 H 1058

Towards chromatin mimics: DNA self-assembly with linker histone H1 - a combined study of X-rays and microfluidics — •Adriana Cristina Toma[1], Rolf Dootz[2], and Thomas Pfohl[1,2] — [1]Chemistry Department, University Basel, Klingelbergstrasse 80, Basel, Switzerland — [2]Max Planck Institute of Dynamics and Self-Organization, Göttingen, Germany

Inspired by the nature of DNA packing we have investigated how linker histone H1influence the local structures of the formed DNA self-assemblies. Despite the key role of the linker-histone H1 in chromatin dynamics, its interactions with nucleosomal DNA are not fully understood. We have used the combination of in situ microfluidics and small angle X-ray microdiffraction in order to analyze the real-time dynamics and structural evolution of assemblies resulted from the binding of linker-histones H1 to DNA. Our results indicate that the mechanism of H1 interactions with DNA is a two-step process: at first H1 binds non-specifically to DNA and secondly the protein molecules rearrange inside the formed self-assemblies, distorting the columnar phase of DNA.

BP 13.10 Wed 12:15 H 1058

The partially closed conformation of DNA polymerase I provides a decision point for nucleotide selection — •Johannes Hohlbein[1], Catherine Joyce[2], and Achillefs Kapanidis[1] — [1]Biological Physics Research Group, Dept. of Physics, University of Oxford, UK — [2]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, U.S.A.

The high fidelity of many DNA polymerases depends largely on conformational changes that precede the chemical step of phosphoryl transfer and serve as checkpoints to reject inappropriate substrates early in the reaction. One of these conformational changes is the fingers-closing transition, during which the fingers subdomain moves from an open to a closed conformation.

Here, we use single-molecule FRET to resolve conformational changes within the bacterial DNA polymerase I with sub-nanometre resolution. We compared the wild-type polymerase to derivatives bearing single amino-acid substitutions at residues E710 and Y766, both which are invariant within the A family of DNA polymerases.

Our results show that these derivatives have decreased affinity for the complementary dNTP, and do not perform efficient fingers-closing. Instead, intermediate FRET states are populated, which are likely to correspond to a fidelity-associated partially closed state of the fingers.

These differences in the interactions and conformations formed along the reaction path reduce discrimination between complementary and non-complementary nucleotides, and provide a basis for the reduced fidelity of the derivatives.

BP 13.11 Wed 12:30 H 1058

Stability of double-stranded oligonucleotide DNA with a bulged loop: a microarray study — •Christian Trapp — Universität des Saarlandes, Biologische Experimentalphysik

The hybridization process, the formation of the DNA double-helix from single-strands of complementary sequences, is important for all living organisms. It is at the basis of many high throughput nucleic acid based technologies such as high throughput sequencing or DNA microarrays. The latter consist of surface bound ssDNA (probes), which can selectively bind to complementary strands (targets) in solution resulting in highly parallel measurements. The underlying physical mechanisms of the hybridization process are poorly understood. We have shown that the binding to DNA microarrays can be easily modeled when the length of probe and targets match [1-2]. Here we investigate the binding of longer targets to microarrays, which hybridize to the probes forming bulged loops. We systematically vary loop position and loop size and show that the result can be reproduced with simple theoretical models at thermal equilibrium, which also apply to solution-phase experiments.

[1] Naiser T, Kayser J, Mai T, Michel W, Ott A: Stability of a Surface-Bound Oligonucleotide Duplex Inferred from Molecular Dynamics: A Study of Single Nucleotide Defects Using DNA Microarrays, Phys. Rev. Lett. 2009, 102, 218301

[2] Naiser T, Kayser J, Mai T, Michel W, Ott A: Position dependent mismatch discrimination on DNA microarrays - experiment and Model, BMC Bioinformatics 2008, 9:509

BP 13.12 Wed 12:45 H 1058

Nucleobase adsorbed at graphene devices: enhance biosensorics — •Bo Song[1], Gianaurelio Cuniberti[2], Stefano Sanvito[3], and Haiping Fang[1] — [1]Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, P.O. Box 800-204, Shanghai 201800, China — [2]Institute for Materials Science and Max Bergmann Center of Biomaterials, Dresden University of Technology, 01062 Dresden, Germany — [3]School of Physics and CRANN, Trinity College, Dublin 2, Ireland

Graphene as a good material for sensing single small molecules, is hardly believed to identify bio-molecules via electrical currents. This is because bio-molecules tend to bind to graphene through non-covalent bonds, such as π-π stacking interaction, which is not customarily considered to induce a clear perturbation of the graphene electronic structure. In contrast to these expectations, we demonstrate that oxygen in nucleobases adsorbed on graphene with π-π stacking interaction, can clearly alter the electric current, even in water at room temperature. This property allows us to devise the strategies employing graphene as material of choice in bio-sensorics, bio-chips.