Topical Talk

BP 13.1 Wed 9:30 H 1058
Chemo-mechanics of a ring-shaped helicase during unwinding
— Michael Schlüer1,2, Gang Sang Wang3, Xiaohuang Chen1, and Taekjip Ha1,4 —
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Most replicative helicases are hexameric ring-shaped enzymes and are essential for cell survival. Despite extensive biochemical, biophysical, 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 ultra-weak 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 Valeriani, 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 Reith1, Andrei Stasiak2, Peter Cifra3, and Christof Schöpflin2 — 1 Department of Physics, Uni Mainz — 2 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 Wagner1, David Singer1, Gang Sang Wang2, and Christof Schöpflin1 — 1 Department of Physics, Uni Mainz — 2 Center for Integrative Genomics, UNIL, Lausanne, Switzerland

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 X0 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.


BP 13.5 Wed 10:45 H 1058
Electrophoretic mobility of DNA-grafted single colloids as studied by optical tweezers — Ilna Semenova, Christoph Gutsche, Mahdy M. Elmahdy, Olaf Überschär, and Friedrich Kremmer — 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 mM/1 - 1 M M/1) and valency (KCl, CaCl2, LaCl3) of the ions in the surrounding aqueous medium, as well as 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 DNA-grafted single colloid at high ionic strength can be understood quantitatively within the limits of the linearized Poisson-Boltzmann equation.


BP 13.6 Wed 11:00 H 1058
RNA folding dynamics studied with a structure-based model — Michael Faber and Stephan Klump — 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 Müller1, Robert Schöpflin1, Nina Krehl2, Radka Ettlinger2, Jörg Schnier1, and Gero Wiedemann1,2 — 1 CCG Bioinformatics, University of Applied Sciences Stralsund, Zur Schwedenhansschanz 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
Confinement Driven Spatial Organization of Semiflexible repositioned nucleosome revealed an increased kinking susceptibility. Moreover, the region proximate to a which is larger than thermal fluctuations but within the range of ATP-dependent biological processes. We repositioning nucleosomes by a range of base pairs. After simulation, we have used the combination of in situ microfluidics and small dynamics, its interactions with nucleosomal DNA are not fully understood. We have shown that the binding to DNA microarrays can be easily modulated 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.

Towards chromatin mimics: DNA self-assembly with linker histone H1 - a combined study of X-rays and microfluidics — Adriana Cristina Toma1, Rolp Dootz2, and Thomas Pföh1,2 — Chemistry Department, University Basel, Klingelbergstrasse 80, Basel, Switzerland — 2Max Planck Institute of Dynamics and Self-Organization, Göttingen, Germany

Inspired by the nature of DNA packing we have investigated how linker histone H1 influence the local structures of the formed DNA self-assemblies. Despite the role of 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, distort the columnar phase of DNA.

The partially closed conformation of DNA polymerase I provides a decision point for nucleotide selection — Johannes Hohleim1, Catherine Joycr2, and Achilleps Kapanidis3 — 1Biological Physics Research Group, Dept. of Physics, University of Oxford, UK — 2Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, U.S.A.

The high fidelity of DNA polymerases depends largely on conformational changes that precede the chemical step of phosphohyl 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 of 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.