BP 44: DNA/RNA and related enzymes

Time: Thursday 15:00–17:00 Location: H 1058

Invited Talk BP 44.1 Thu 15:00 H 1058

Molecular Systems Engineering with DNA: Four pieces, one rule, and many possibilities. — •Hendrik Dietz — Technische Universität München, Munich, Germany

It is notoriously difficult to observe, let alone control, the position and orientation of molecules because of their small size and the constant thermal fluctuations that they experience in solution. Molecular self-assembly with DNA provides a route for placing molecules and constraining their fluctuations in user*defined ways, thereby opening attractive and unprecedented avenues for scientific and technological exploration. In my talk I will introduce some of the key concepts and methods, and highlight a number of recent applications.

BP 44.2 Thu 15:30 H 1058

Mechanisms of backtrack recovery by RNA polymerases I and II — •Ana Lisica[1,2], Marcus Jahnel[1,2], Christoph Engel[3], Edgar Roldan[4,5], Patrick Cramer[3], and Stephan Grill[1,2,4] — [1]BIOTEC, Dresden, Germany — [2]MPI CBG, Dresden, Germany — [3]MPI PBC, Göttingen, Germany — [4]MPI PKS, Dresden, Germany — [5]GISC, Madrid, Spain

RNA polymerases (Pol) backtrack frequently during transcription elongation. To recover from the backtracked state, Pol I uses a strong transcript cleavage activity, while that of Pol II is weak but can be enhanced by transcription factor TFIIS. However, backtrack recovery can also proceed by 1D diffusion, and the mechanisms that underlie the choice of backtrack recovery pathway have not been investigated. Here, we use dual-trap optical tweezers to compare Pol I and Pol II transcription and backtrack dynamics. We find that Pol I is faster than Pol II, pauses less often, and can transcribe against higher opposing forces. Neither enzyme can recover alone from backtracks beyond a threshold depth, and only Pol II with TFIIS can rapidly recover from deep backtracks. Comparing recovery times from varying backtrack depths with expectations from the theory shows that the choice of backtrack recovery pathway is determined by a kinetic competition between 1D diffusion and transcript cleavage. In Pol I, this balance is influenced by the TFIIS-homologous subunit A12.2, which both decreases the rate of 1D diffusion and increases the rate of cleavage. Our data identifies the distinct backtrack recovery behaviours of Pol I and Pol II that evolved to serve specific cellular roles of these enzymes.

BP 44.3 Thu 15:45 H 1058

Fast Chromatin Assembly facilitated by Nucleosome Breathing and Replication-Guided Packing — •Johannes Nuebler[1], Brendan Osberg[1], Philipp Korber[2], and Ulrich Gerland[1] — [1]Theory of Complex Biosystems, Physik-Department, Technische Universität München, James-Franck-Str. 1, 85748 Garching, Germany — [2]Adolf-Butenandt-Institut, University of Munich, Schillerstrasse 44, 80336 Munich, Germany

The condensation of eukaryotic DNA into chromatin entails the formation of dense nucleosome arrays. These arrays are frequently destroyed by transcription and replication, such that reassembly is required. Due to a jamming effect in the random adsorption of mutually exclusive objects (the 'car parking problem'), the question was raised how *in vivo* nucleosome densities, and patterns, can be reached in the biologically relevant timescale of minutes [1]. We show that the 'softness' of nucleosomes alleviates this kinetic challenge [2]. Nucleosome softness arises due to transient DNA unwrapping (breathing) and stepwise nucleosome assembly. From a physics perspective, the 'soft car parking problem' differs fundamentally from its hard counterpart by exhibiting non-monotonic density and rapid equilibration. We also discuss scenarios how the progression of the replication fork can promote rapid reassembly in its wake. For example, tight packing arises naturally if the fork progresses slowly compared to the reassembly rate.

[1] R. Padinhateeri, J.F. Marko, PNAS 108, 7799 (2011).

[2] B. Osberg, J. Nuebler, P. Korber, U. Gerland, Nucleic Acids Res. doi: 10.1093/nar/gku1190 (2014)

BP 44.4 Thu 16:00 H 1058

Gene regulation on the RNA level. The B12 dependent btuB riboswitch studied with single molecule FRET. — •Richard Börner, Michelle Schaffer, Sofia Gallo, and Roland K.O. Sigel — Department of Chemistry, University Zurich, Switzerland

The btuB riboswitch is one of the promising candidates for understanding the gene regulation at the RNA level (1). This B12 specific RNA is encoded in the 5'-untranslated region (UTR) of the btuB gene encoding a coenzyme B12 (AdoCbl) transporter found among other bacteria, especially in E. coli. Upon the binding of B12, a conformational switch of the btuB aptamer occurs, thus inhibiting the expression of the cellular B12 transporter. Although studied intensively on a bulk level (2), the kinetics and in particular the exact structural information of this riboswitch is still missing, as neither a crystal nor a NMR structure exists.

Herein, we use Förster resonance energy transfer on a single molecule level (smFRET) as a versatile tool to characterize the conformational states and the folding kinetics of the aptamer region of the btuB riboswitch. Thereby, we will especially focus on the influence of AdoCbl and the function of Mg2+ for folding and switching. As FRET is known to be used as molecular ruler, we are aiming at absolute rather than apparent distance measurements (3). Thus, a study of the global structure of the btuB riboswitch will complement our experiments.

1. Perdrizet, G. A., et al. 2012. Proc. Natl. Acad. Sci. U. S. A. 109:3323-3328. 2. Choudhary, P. K. and R. K. Sigel. 2014. RNA 20:36-45. 3. Sindbert, S., et al. 2011. J. Am. Chem. Soc. 133:2463-2480.

BP 44.5 Thu 16:15 H 1058

Random association of neighbouring replicons creates DNA replication factories. — •Jens Karschau[1,2], Nazan Saner[3], Toyaki Natsume[3], Renata Retkute[4], Conrad A. Nieduszynski[5], J. Julian Blow[3], Alessandro P.S. de Moura[2], and Tomozuki U. Tanaka[3] — [1]MPI PKS, Dresden, Germany — [2]University of Aberdeen, U.K. — [3]University of Dundee, U.K. — [4]University of Nottingham, U.K. — [5]University of Oxford, U.K.

For simplicity, cartoons often depict DNA replication on a straight 1D line. In fact, we deal with a polymer that is packed and modified on different levels yielding higher order structures of organisation. Processing a DNA piece (as for example during DNA synthesis in clusters of replication factories) requires proper coordination amongst all individual machines (replicons) on it. However, it remains unknown how such replicons are organised at each replication factory.

We apply a 2 bead on a string model for two neighbouring replicons. We calculate analytically the probability for replicons to meet using Boltzmann statistics and then fit this with experimental data of replicon association in yeast to determine binding energies. This suffices to link our model to the dynamics of replicon activation and movement along DNA during the synthesis phase, to extrapolate from 2 neighbour interactions to the whole yeast-genome, and to describe properties of measured experimental distributions of fork numbers per cluster. Our model yields a near perfect match with the data suggesting that actively replicating units of DNA randomly associate with each other to form replication factories rather than being controlled by the cell.

BP 44.6 Thu 16:30 H 1058

Vibrational dynamics and hydration of the DNA backbone — •Biswajit Guchhait, Yingliang Liu, Rene Costard, Torsten Siebert, and Thomas Elsaesser — Max-Born-Institut für Nichtlinear Opitk und Kurzzeitspektroskopie, Max-Born-Str. 2a, 12489 Berlin, Germany

The DNA double helix and its aqueous environment display structural dynamics on the ultrafast time-scale of molecular motions. Two-dimensional (2D) infrared spectroscopy is employed in a frequency range from 950 to 1300 cm^{-1} to map and discern the vibrational excitations of the fully hydrated DNA backbone, their couplings and the interactions with the surrounding water shell. The mutual couplings and corresponding delocalized nature of vibrational modes of the phosphate group, phosphodiester linkage and furanose ring structure within the backbone are evident from the rich cross peak pattern observed in the 2D spectra. The couplings further facilitate a picosecond transfer of vibrational energy from the phosphate to the sugar linkage and ring modes. The phosphate modes further play a key role for the interaction with the surrounding water shell, where structural fluctuations of interfacial water are limited and slowed down compared to bulk H_2O. This behavior is attributed to steric hindrance and the action of strong electric fields at the interface.

BP 44.7 Thu 16:45 H 1058

Continuous, sequence dependent gelation of nucleic acids driven by a thermal gradient. — •Christof Mast, Matthias Morasch, and Dieter Braun — Systems Biophysics, Faculty of Physics, LMU Munich, Amalienstrasse 54, 80799 Munich

Under equilibrium conditions, the phase transition of bio-polymers like DNA toward a compacted state requires mutual binding forces from high polymer concentrations or multivalent ions. We demonstrate that the physical non-equilibrium of a thermal gradient across an elongated chamber accomplishes this task from dilute DNA solutions without the help of multivalent ions, proteins, evaporation or encapsulation. The gelation process is governed by the base pair interactions between respective DNA strands, leading to a highly nonlinear sequence dependency of the gelation process. DNA of different sequences are compacted at distinct sites, yielding a sequence-based physical sorting mechanism. DNA strands with low hybridization energies do not form a gel within the measurement time. The DNA gel is continuously rebuilt inside the thermal gradient in a dynamic turn-over fashion and remains stable for days under dilute equilibrium conditions. The process implements a basic prebiotic machine that selects and stores sticky sequence motifs out of a random sequence pool.