BP 20: DNA, RNA and Chromatin

Time: Thursday 14:00–17:15 Location: HÜL 186

Invited Talk BP 20.1 Thu 14:00 HÜL 186

Artificial biochemical reaction circuits based on DNA and RNA — •Friedrich Simmel, Eike Friedrichs, and Ralf Jungmann — Physik Department E14, TU München

Hybridization between complementary sequences of DNA or RNA combined with production and controlled degradation of RNA regulatory molecules can be used for the construction of simplified analogues of naturally occurring biochemical reaction circuits. Such circuits can be used to employ logical decisions, but also interesting dynamical behavior such as oscillations or bistability into biochemical systems. This can be applied, e.g., to control the motion of molecular devices or the synthesis of functional RNA molecules such as RNA aptamers. Experimentally, the dynamical behavior of these reaction networks is studied in bulk solution (in vitro), but also in small reaction compartments such as lipid vesicles or microemulsion droplets. Experiments are supported by numerical studies using deterministic and stochastic models of the networks.

BP 20.2 Thu 14:30 HÜL 186

Self-assembling DNA-caged particles: nanoblocks for hierarchical self-assembly — •Nicholas Licata[1,2] and Alexei Tkachenko[1] — [1]University of Michigan, Ann Arbor, USA — [2]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

DNA is an ideal candidate to organize matter on the nanoscale, primarily due to the specificity and complexity of DNA based interactions. Recent advances in this direction include the self-assembly of colloidal crystals using DNA grafted particles. In this talk we theoretically discuss the self-assembly of DNA-caged particles. These nanoblocks combine DNA grafted particles with more complicated purely DNA based constructs. Geometrically the nanoblock is a sphere (DNA grafted particle) inscribed inside a polyhedron (DNA cage). The faces of the DNA cage are open, and the edges are made from double stranded DNA. The cage vertices are modified DNA junctions. We calculate the equilibrium yield of self-assembled, tetrahedrally caged particles, and discuss their stability with respect to alternative structures. The experimental feasability of the method is discussed. To conclude we indicate the usefulness of DNA-caged particles as nanoblocks in a hierarchical self-assembly strategy.

BP 20.3 Thu 14:45 HÜL 186

Images of Intracellular Kinetics Reveal Accellerated DNA Hybridization — •Ingmar Schön and Dieter Braun — Systems Biophysics, LMU München, Germany

Molecular crowding affects the diffusion properties and the free energies of molecules in densely packed environments. Its impact on reaction kinetics in the relevant context of living cells is still elusive, mainly due to the difficulty of capturing fast kinetics *in vivo*. In this talk, we show spatially resolved measurements of DNA hybridization kinetics in single living cells. HeLa cells were transfected with a FRET labeled dsDNA probe by lipofection. We characterize the reaction kinetics at each image pixel with a kinetic range of $10^{-5} \dots 10^{0}$s by combining laser-driven temperature oscillations, stroboscopic illumination, fluorescence imaging, and frequency-based relaxation analysis. Within individual cells and between different cells, the time constant of the reaction varied according to different DNA concentrations. A quantitative analysis of the concentration dependence revealed that the association rate was considerably enhanced compared to free solution, likely due to molecular crowding effects inside the cell. The imaging modality of our technique facilitates the parallel measurement of different cellular compartments such as the cytoplasm, the nucleoplasm or even the nucleoli. In general, our technique which we call TOOL (Temperature Oscillation Optical Lock-in) microscopy opens up the possibility to map cellular differences in the reaction environment on the micrometer scale and provides quantitative data about intermolecular kinetics for systems biology.

BP 20.4 Thu 15:00 HÜL 186

Promoter proximal transcript secondary structure — •Abigail Klopper and Stephan Grill — Max Planck Institute for the Physics of Complex Systems, Dresden

RNA polymerase transcribes selected parts of the DNA genome into RNA transcripts by advancing processively along a double-stranded DNA template. It melts the DNA into a single-stranded bubble and catalyzes bond formation, which effectively polymerizes the complementary RNA strand. There is evidence to suggest that the nascent RNA forms self-interacting secondary structure elements. These are thought to serve as barriers to an inactive backtracked state, aiding recovery to an active conformation and ensuring the timely production of a functional transcript. We investigate the role of conformational characteristics of the RNA strand in the context of the early stages of transcription, during which the polymerase is prone to premature and irreversible stalling. Specifically, we examine the hypothesis that the absence of long transcripts is the primary cause of stalling in the vicinity of the promoter. Despite prolific attention paid to the conformational statistics of long RNA strands, little is understood about the implications of finite size in shorter strands. With a recursive formulation of the partition function for homogeneous and disordered RNA molecules, we utilize numerical and analytical approaches to calculate the average number of unpaired bases adjacent to the polymerase. We find that the length-dependent equilibrium fold attributed to the nascent strand poses a marked barrier to a backtracking polymerase within length scales commensurate with early stalling events.

BP 20.5 Thu 15:15 HÜL 186

overstretching of DNA duplexes studied with steered molecular dynamics simulations — •hui li and thomas gisler — Universität Konstanz, Fachbereich Physik, 78457 Konstanz, Germany

Single-molecule experiments on long-chain DNA show that the molecule can be overstretched at nearly constant force (65-110 pN) to 60% beyond its relaxed contour length. The origin of this plateau in the force-extension curve is still under debate. Molecular dynamics (MD) simulations of a short DNA duplex with 12 base pairs suggest that it is caused by a transition to a new conformation ("S-DNA") with inclined base pairs; competing scenarios based on MD simulations of 12-basepair DNA duplexes and thermodynamic considerations suggest the force plateau to arise from a continuous loss of base pairing under external force.

In this contribution we present results from steered MD simulations of a 30-basepair DNA duplex for which a force plateau was recently observed experimentally. We analyzed the pairing between complementary bases and the tilt angles during the stretching. Structure at the force plateau show a coexistence of "S-DNA" segments with "B-DNA" segment which are separated by denatured bases, which has not been seen in stretching simulations of shorter oligomers. In addition, we study the effect of a mismatched base or a gap in backbone on the force plateau and stretched structure. A mismatched base has little effect on the force curves, but the neighbor segments are unwound at the force plateau. A gap in backbone causes the "nicked" region denatured and stretched more than other parts.

BP 20.6 Thu 15:30 HÜL 186

Modeling the BS-transition of DNA under tension — •Thomas Rudolf Einert[1], Douglas Staple[2,3], Hans-Jürgen Kreuzer[2], and Roland Netz[1] — [1]Physik Department, Technische Universität München, 85748 Garching b. München, Germany — [2]Department of Physics and Atmospheric Science, Dalhousie University, Halifax, NS B3H 3J5 Canada — [3]Max-Planck-Institut für Physik komplexer Systeme, 01187 Dresden, Germany

Stretching of double-stranded DNA leads to the denaturation of the molecule. A stretching force $F \approx 65\,\mathrm{pN}$ induces a sharp, structural transition where DNA changes from its native state (B-DNA) to a stretched state (S-DNA). At even higher forces the hydrogen bonds break up and loops start to form giving rise to a second, smoother transition.

We present two statistical mechanics models which exhibit both transitions. To describe the behavior of DNA under tension we use the worm-like chain (WLC) model. Our formulation allows to give arbitrary length-dependent weights for loops. Force-extension curves can be calculated analytically and show excellent agreement with experimental data. In the thermodynamic limit genuine phase transitions are possible depending on the parameterization of the three different states (B-DNA, S-DNA, or loops). The phase transitions are characterized by their order and critical exponents.

15 min. break

BP 20.7 Thu 16:00 HÜL 186

An inter-nucleotide potential for DNA: atomistic and coarse-grained simulations — •Maria Fyta[1,2], Greg Lakatos[1], Simone Melchionna[1], and Efthimios Kaxiras[1] — [1]Department of Physics and School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA — [2]Present address: Physics Department, Technical University of Munich, Germany

The structural and dynamical properties of double stranded DNA play a critical role in range of fundamental biological and technological processes. Such processes include DNA translocation through both artificial and nuclear nanopores, the wrapping of DNA around histone proteins, and the use of DNA molecules as nanotethers in a variety of nanoscale devices. To understand the behavior of DNA in these contexts, it is desirable to have a computational model capable of treating oligomers with hundreds to thousands of base pairs, on time scales of microseconds or longer. Utilizing accurate density-functional electronic structure techniques, we are developing a coarse-grained molecular model of double stranded DNA (dsDNA) capable of reproducing the molecule's structural and dynamical properties on these length and time scales. Initial validations of the model indicate that it reproduces a number of experimentally measured structural features of DNA, including the persistence length under physiologic conditions. The model resulting from this study will be used to investigate the behavior of dsDNA during nanopore translocation, and the response of dsDNA to mechanical loads.

BP 20.8 Thu 16:15 HÜL 186

Investigation of the three-dimensional structure of chromatin — •René Stehr[1], Nick Kepper[2], Ramona Ettig[2], Karsten Rippe[2], and Gero Wedemann[1] — [1]Fachhochschule Stralsund, System Engineering and Information Management, 18435 Stralsund, Germany — [2]Deutsches Krebsforschungszentrum & BioQuant, Research Group Genome Organization & Function, 69120 Heidelberg, Germany

We developed a new coarse-grained computer model of chromatin, which enhances the common two-angle model by additional four angles and uses a new nucleosome-nucleosome interaction potential. Based on recent experimental data of native and reconstituted chromatin, three models of chromatin fibers were systematically analyzed by Monte Carlo simulations [1,2]. The results indicate the strong influence of the nucleosome repeat length on the stability of the fiber formation. A model was proposed, in which changes of the chromatin fiber conformation induced by linker histone H1 binding are reproduced by relatively small changes of the local nucleosome geometry. Furthermore, key factors for the control of compaction and higher order folding of the chromatin fiber were identified. We have further developed this approach and are applying it to the analysis of the conformational space of the chromatin fiber, fiber force spectroscopy experiments and atomic force microscopy imaging of chromatin fibers.

[1] Stehr, R., N. Kepper, K. Rippe, and G. Wedemann. Biophys. J. 95:3677 (2008).

[2] Kepper, N., D. Foethke, R. Stehr, G. Wedemann, and K. Rippe. Biophys. J. 95:3692 (2008).

BP 20.9 Thu 16:30 HÜL 186

Structural levels of organization in the TmHU/DNA-complex as studied by optical tweezers assisted force spectroscopy — •Carolin Wagner, Mathias Salomo, and Friedrich Kremer — Universität Leipzig, Germany

The interaction of the histone-like protein TmHU (from Thermotoga maritima) to DNA is analyzed on a single molecule level by use of optical tweezers. This technique provides a nm-resolution in positioning a micron-sized colloid and an accuracy of +/-50 fN in measuring the forces acting on it. As a further refinement, our set-up is now accomplished with a fast feed-back loop (regulation frequency: 30 Hz) which allows to carry out the experiment under conditions of a constant and adjustable force.

The proceeding of the condensation and its dependence on the applied force (2-40 pN) is investigated. At a pre-stretching of 2 pN the length of the DNA is reduced by about 80%. At higher forces, the reaction is disrupted at an incomplete level. The process shows two distinct regimes that can be related to different organizational levels. The condensation also shows a pronounced dependence on the concentration. By stretching the TmHU/DNA-complex, it is possible to disrupt the proteins from the DNA. The length of the smallest event conforms with the results of a simulated rupture.

BP 20.10 Thu 16:45 HÜL 186

Extracting intermolecular forces in protein-DNA complexes from structural data — •Nils Becker and Ralf Everaers — Laboratoire de Physique, École Normale Supérieure, Université de Lyon

It is a standard exercise in mechanical engineering to infer external forces acting on a body, when given its shape and elastic properties. We apply this kind of analysis to distorted double-helical DNA in complexes with proteins, and extract the local *mean* forces and torques acting on each base-pair of bound DNA from high-resolution complex structures. The analysis relies on known elastic potentials and a careful choice of coordinates for the well-established rigid base-pair model of DNA. The results reveal the complex nano-mechanical patterns of interaction between proteins and DNA. An application of this idea to 146bp and 147bp crystal structures of the nucleosome core particle reveals a characteristic force pattern at the well-known DNA contact sites, and leads to an explanation of twist defect placement in the irregular 146bp structure.

BP 20.11 Thu 17:00 HÜL 186

Physical Analysis of Statistical Nucleosome Positioning in the Yeast Genome — •Wolfram Möbius[1,2] and Ulrich Gerland[1,2] — [1]Institute for Theoretical Physics, Universität zu Köln — [2]Arnold Sommerfeld Center and Center for NanoScience, LMU München

Recent experiments determined nucleosome positions in the yeast genome [1-3] and identified two salient features in the spatial organization: (i) nucleosome-free regions upstream of many transcription start sites (TSS), and (ii) an oscillatory nucleosome density downstream. The mechanisms underlying these patterns are less clear. One possible scenario is that the majority of nucleosome positions near the TSS are directly determined by DNA sequence [4], binding competition with other proteins [5], or by active remodelling. An alternative scenario is that only a minority of these nucleosomes is directly positioned by the DNA sequence, forming barriers which strongly constrain the positions of closeby nucleosomes, purely on statistical grounds [3]. Specifically, the nucleosomes might be seen as a one-dimensional gas of rods (Tonks gas) with a few barriers in between. To quantitatively test this scenario, we assess whether the experimentally observed oscillations in nucleosome occupancy are indeed compatible with Tonks gas statistics. Furthermore, we estimate whether biologically reasonable binding specificity suffices to form barriers able to create nucleosome free regions as observed. [1] G.-C. Yuan et al., Science **309**, 626 (2005) [2] W. Lee et al., Nature Genetics **39**, 1235 (2007) [3] T. Mavrich et al., Genome Research **18**, 1073 (2008) [4] E. Segal et al., Nature **442**, 772 (2006) [5] A.V. Morozov et al., arxiv.org:0805.4017v1 (2008)