BP 2: DNA/RNA and related enzymes

Time: Monday 9:30–13:00 Location: ZEU 250

BP 2.1 Mon 9:30 ZEU 250

Thermal disequilibrium causes natural selection of replicating DNA — •Moritz Kreysing[1,2], Lorenz Keil[1], Simon Lanzmich[1], Christof Mast[1], Stephan Krampf[1], and Dieter Braun[1] — [1]Systems Biophysics, LMU Munich, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics

Here we report on experimental findings that a mere temperature gradient across a sub-millimeter sized compartment can be used to filter bio-molecules from dilute solutions. Because the characteristics of this thermophoretically based filter are strongly non-linear with regard to polymer length, the through-flow system is able to accumulate exclusively long polymers, as firstly shown here by the length selective fractionation of solute DNA strands. Exploiting convectively driven temperature cycles [1], the trapped DNA is additionally able to replicate in a PCR type manner. As we demonstrate, the combination of length selective accumulation and replication renders this compartment a niche in which heterogeneous populations of DNA strands are subject to a selection pressure in favor of molecular complexity, a so far unresolved prerequisite to the onset of Darwinian evolution [2].

References: 1) C. Mast and D. Braun, PRL, 104:188102 (2010), 2) D. Mills, L. Peterson, S. Spiegelman, PNAS, 58:217 (1967)

BP 2.2 Mon 9:45 ZEU 250

Neutral Evolution of Duplicated DNA: An Evolutionary Stick-Breaking Process Causes Scale-Invariant Behavior — Florian Massip[1,2] and •Peter F. Arndt[2] — [1]INRA, Jouy-en-Josas, France — [2]Max Planck Institute for Molecular Genetics, Berlin, Germany

Recently, an enrichment of identical matching sequences has been found in many eukaryotic genomes. Their length distribution exhibits a power law tail raising the question of what evolutionary mechanism or functional constraints would be able to shape this distribution. Here we introduce a simple and evolutionarily neutral model, which involves only point mutations and segmental duplications, and produces the same statistical features as observed for genomic data. Further, we extend a mathematical model for random stick breaking to analytically show that the exponent of the power law tail is -3 and universal as it does not depend on the microscopic details of the model.

BP 2.3 Mon 10:00 ZEU 250

Insights into the thermodynamic profile of "mutated" Cytosine rich DNA strands: A theoretical study — •Vasileios Tatsis and Andreas Heuer — Institut für Physikalische Chemie, Münster, Germany

Cytosine rich DNA sequences form four stranded structures (i-motif) under acidic conditions. The i-motif is an intercalated structure formed by association in a head to tail orientation of two parallel duplexes whose strands are held together by hemiprotonated Cytosine·Cytosine(+) pairs. Our theoretical work examines how point defects in the central strands and variations of the Cytosine sequences' length affect the thermodynamical stability of the i-motif structure. We employ the fully atomistic Molecular Dynamics method with an explicit solvent model. We use as an initial structure for the "mutations" a single stranded deprotonated DNA i-motif. Furthermore, in order to enhance the conformational sampling and to compute the thermodynamic stability of the new "mutated" i-motif structures, we use the Metadynamics and the Steered Molecular Dynamics techniques. The output from this theoretical study is compared with experimental results derived from CD-experiments.

BP 2.4 Mon 10:15 ZEU 250

Extreme polymerization and aggregation of DNA/RNA in thermal traps — •Christof Mast[1], Severin Schink[2], Moritz Kreysing[1], Urlich Gerland[2], and Dieter Braun[1] — [1]Systems Biophysics, Physics Department, Center for Nanoscience, LMU Munich, Germany — [2]Arnold-Sommerfeld-Center for Theoretical Physics and Center for Nanoscience, LMU Munich, Germany

Biopolymers like RNA, DNA and proteins are the fundamental actors in all life on earth. It is however unclear, how the first long RNA polymers with enzymatic activity could arise in a prebiotic scenario: Even in millimolar concentrations, ribonucleic acids only polymerize to short strands with a length of 20 bases. We demonstrate how a simple thermal gradient in a hydrothermal pore-like geometry is able to trap longer polymers exponentially better than shorter polymers. Polymerization leads to even longer polymers due to the increased total mass in the trapping center. Polymerization and thermal trapping are mutually self-enhancing. This process is described by an experimentally supported theory of trapped polymerization. Theoretical extrapolation to RNA-world conditions shows that a pore height of 5 cm and a temperature difference of 10 K are sufficient to form RNA polymers longer than the shortest RNA based replicator. Thermal traps also support the sequence specific formation of large aggregates made by the reversible polymerization of sticky-ended dsDNA. The melting temperature of the aggregates and the sticky ends match. No aggregates were found with non-polymerizing dsDNA pieces or without thermal trapping which therefore acts as a highly sequence selective process.

BP 2.5 Mon 10:30 ZEU 250

Complex RNA folding kinetics revealed by single molecule FRET and hidden Markov models — •Bettina Keller[1], Andrei Kobitski[2], G. Ulrich Nienhaus[2], and Frank Noé[3] — [1]Freie Universität Berlin, Institut für Chemie, Takustr. 3, 14195 — [2]Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany — [3]Freie Universität Berlin, Institut für Mathematik, Arnimallee 6, 14195

We have developed a hidden Markov model and optimization procedure for photon-based single-molecule FRET data, which takes into account the trace-dependent background intensities. This analysis technique reveals an unprecedented amount of detail in the folding kinetics of the Diels-Alderase ribozyme. Depending on the Mg2+ concentration, 7 to 9 states can be distinguished, including putative native, near-native and misfolded states. Some states exist only at either low or high Mg2+ concentrations, while other states exhibit little sensitivity to Mg2+. There is a general tendency for structures to become more compact upon the addition of Mg2+. A hierarchy of timescales was found, including dynamics of 10 ms or faster, likely due to tertiary structure fluctuations, and slow dynamics on the seconds timescale, presumably associated with significant changes in secondary structure. The folding pathways proceed through a series of intermediate secondary structures. There exist both, compact pathways and more complex ones in which structures show tertiary unfolding, then secondary refolding, and subsequently tertiary refolding.

BP 2.6 Mon 10:45 ZEU 250

***In situ* release of DNA from artificial gene carriers** — •Natalja Strelnikova[1], Adriana Toma[1], Rolf Dootz[2], and Thomas Pfohl[1] — [1]University of Basel, Department of Chemistry, Switzerland — [2]Max Planck Institute for Dynamics and Self Organization, Göttingen, Germany

In chromosomes DNA exists in a highly organized state, wrapped around histones, forming a composite material called chromatin. Decondensation of DNA plays an essential role in gene expression. DNA must be unpacked for transcription, arousing interest in the understanding of underlying interaction mechanisms of DNA decompaction. Our goal is to discover the real-time dynamics of the release of DNA from artificial gene carriers (dendrimer PAMAM 6/DNA). We employ a newly developed soft lithography-based microfluidics reaction device in combination with a lab source X-ray instrument. Using SAXS and SAXD (small angle X-ray scattering and diffraction), we can study the nanostructural evolution of the involved processes. We observe the dynamics of DNA release from the gene carrier using heparin as an anionic competitor. Here, negatively charged heparin competes with phosphate groups of DNA to interact with positively charged amines of PAMAM 6. The impact of the heparin and the salt concentrations as well as the pH on the disintegration of DNA/PAMAM 6 complexes can be analyzed in a temporal manner.

15 min. break

Topical Talk BP 2.7 Mon 11:15 ZEU 250

Single molecule torque and twist measurements probe the key players of the central dogma — •Jan Lipfert — Department of Physics and CeNS, University of Munich, Germany

Single-molecule manipulation techniques have provided unprecedented

insights into the structure, function, interactions, and mechanical properties of biological macromolecules. While many single-molecule manipulation techniques naturally operate in the space of (linear) extension and force, recently a number of techniques have been developed that enable measurements of rotation angle and torque. These new methods provide exciting opportunities to probe biological important macromolecules. In particular, the helical nature of double-stranded DNA and RNA intrinsically links key processes such as replication, transcription, and genome repair to rotational motion and the accumulation of torsional strain.

In my talk, I will briefly review novel magnetic tweezers assays that enable direct measurements of single molecule torque and twist, notably magnetic torque tweezers (MTT) and freely-orbiting magnetic tweezers (FOMT). Using these techniques, we have for the first time mapped out the complete force-torque phase diagram for double-stranded RNA, discovering some similarities but also striking differences to its better studied cousin, DNA. In addition, I will briefly describe results on Rad51-DNA filaments, a key intermediate in DNA repair, and applications of our novel magnetic tweezers techniques to probe nucleosome dynamics.

BP 2.8 Mon 11:45 ZEU 250

Hybrid Single-Molecule-FRET-Magnetic Tweezers Probe Force-Dependent Conformational Space — •Marko Swoboda, Maj Svea Grieb, Stephan Friebe, Steffen Hahn, and Michael Schlierf — B CUBE, TU Dresden, Arnoldstraße 18, 01307 Dresden, Germany

Single-molecule methods are often separated in force-based and fluorescence-based techniques. Single-molecule Förster resonance energy transfer (smFRET) has proven to be a powerful tool to probe distance changes on the nanometer scale. The technique therefore perfectly matches biomolecular dimensions but lacks the ability to manipulate molecules. Magnetic tweezers are a prime tool to study the mechanics of DNA and its interacting enzymes but their spatial resolution is limited to the direction of force and torque application.

Here, we demonstrate a hybrid instrument, combining smFRET and magnetic tweezers. This allows us to simultaneously probe conformational dynamics in a molecular system, using both force and distance information. We demonstrate the instrument's capabilities by studying the behavior of a fluorescently labeled DNA undergoing rapid conformational fluctuations.

BP 2.9 Mon 12:00 ZEU 250

Probing the kinetics of a model helicase-nuclease with a temperature-controlled Magnetic Tweezers — •Benjamin Gollnick[1], Carolina Carrasco[1], Francesca Zuttion[1], Neville S. Gilhooly[2], Mark S. Dillingham[2], and Fernando Moreno-Herrero[1] — [1]Centro Nacional de Biotecnología, CSIC, Darwin 3, Campus de Cantoblanco, 28049 Madrid, Spain — [2]School of Biochemistry, Medical Sciences Building, University of Bristol, University Walk, Bristol BS8 1TD, UK

Motor protein activities such as ATP hydrolysis and translocation are temperature-dependent; by studying their kinetics under different thermal conditions one can estimate the associated physicochemical parameters. Here, we present a temperature-controlled Magnetic Tweezers setup that allows us to perform single-molecule experiments at temperatures in solution of up to 40 °C with a precision of 0.1 °C. Using this instrument we have been able to compare the translocation activity of individual copies of the bacterial DNA helicase-nuclease complex AddAB - an enzyme involved in the initial steps of double-stranded DNA break repair by homologous recombination - at different thermal settings with results obtained from ensemble measurements. Interestingly, although the two complementary approaches give rise to a systematic difference between their corresponding velocities measured at each temperature, they yield almost identical estimates of the kinetic barrier of the translocation process, which turns out to be on the order of 21 kT and hence similar to activation energies observed for other translocating proteins.

BP 2.10 Mon 12:15 ZEU 250

RNA folding dynamics studied with structure based models — •Michael Faber and Stefan Klumpp — Max Planck Institute of Colloids and Interfaces Potsdam-Golm Science Park; Am Mühlenberg 1 OT Golm; 14476 Potsdam; Germany

RNA molecules form well defined three-dimensional structures on physiologically relevant timescales which are crucial for their enzymatic activity. Already during transcription structured RNA can have regulatory functions. Key to RNA folding is the formation of intramolecular base pairs which is called the secondary structure. We have developed a structure-based model of RNA and use a kinetic Monte-Carlo method to study the dynamics of secondary structures. We apply our methods to the folding and unfolding of several RNA structures in the presence and absence of external load forces. To study the effect of transcription on folding we look at co-transcriptional folding of riboswitches.

BP 2.11 Mon 12:30 ZEU 250

Kinetics of conformational fluctuations in DNA hairpin-loops in crowded fluids — •Olivia Stiehl and Matthias Weiss — Experimentalphysik I, Universität Bayreuth, Germany

Biochemical reactions in crowded solutions are commonly anticipated to differ strongly from those in dilute solutions. Macromolecular crowding not only induces excluded-volume interactions with surrounding molecules but it has also been frequently reported to initiate anomalous diffusion (subdiffusion). Both facets are supposed to stabilize complex formation of reaction partners.

A typical biochemical reaction is the thermally driven opening and closing transition of DNA hairpin-loops. Using fluorescence correlation spectroscopy (FCS), we have revealed that crowding not only slows down the opening/closing kinetics but also increases the steady-state fraction of the closed state significantly [1]. Our results also show that subdiffusion leads to an even stronger shift of the two-state equilibirum towards the closed state in comparison to pure excluded-volume effects. For that reason, we conclude that biochemical reactions are sensitive to both, excluded-volume interactions and changes of the diffusive behavior of the reactants.

Preliminary UV-absorption data also support the notion that the simplification of a two state model is no more justified in crowded solutions as soon as the DNA strand exceeds a certain length.

[1] O. Stiehl, K. Weidner-Hertrampf and M. Weiss: Kinetics of conformational fluctuations in DNA hairpin-loops in crowded fluids. New J. Phys. 15 (2013) 113010.

BP 2.12 Mon 12:45 ZEU 250

The car–parking model solves the random completion problem of DNA replication — •Jens Karschau[1,2], Peter J. Gillespie[3], J. Julian Blow[3], and Alessandro P. S. de Moura[1] — [1]University of Aberdeen, Aberdeen, U.K. — [2]MPI PKS, Dresden, Germany — [3]University of Dundee, Dundee, U.K.

Eukaryotic cells have a large yet fixed amount of replication starting points — origins of replication — whose distance amongst them gives the time to synthesise a DNA segment, and the largest distance ultimately dictates when the last remaining segment is fully synthesised so that a cell is ready to divide.

A naive assumption would be to have origins equally separated from each other to partition the DNA into small segments, so to have minimal replication time. In a model for proteins finding origin positions randomly we show how these origin positions are taken as a result from the spatial requirement for proteins to bind stably at random positions. This explains experimental data of protein-DNA adsorption kinetics showing saturation over time. In a second step, this leads to a problem in statistical physics known as the car-parking problem. A model akin to this successfully explains the criteria to have small segments, because during protein adsorption it is more likely for origin-forming proteins to land in large empty regions on DNA. With our model we solve a long-standing conundrum: how to have optimal origin spacing when adsorption occurs at random sites, i.e. the random completion problem. Its solution directly emerges from physical principles of our adsorption model.