BP 3: DNA, RNA and Associated Enzymes

Time: Monday 14:00–16:45 Location: H43

Invited Talk BP 3.1 Mon 14:00 H43

Exciting positional control with DNA Origami: Onwards nanoscale gadgets for Science and Technology. — •Hendrik Dietz — Laboratory for Biomolecular Nanotechnology, Physik Dept, Technische Universität München - Garching, Germany

Scaffolded DNA Origami (1) is a molecular self-assembly method that enables folding a multiple-kilobase 'back-bone' DNA molecule into complex nanoscale shapes by introducing interactions between different segments on the backbone molecule. Interaction patterns are expressed by sets of synthetic 'staple' molecules that are added to the much longer back-bone molecule. Based on this concept we have developed a general approach to the construction of custom three-dimensional shapes that can be conceptualized as creating custom-crossection bundles of DNA double helices (2) where the number, arrangement, and lengths of helices can be freely designed. We further enabled building yet more sophisticated shapes that also twist and bend in desired ways (3). Importantly, DNA origami retains spatial registry over each of thousands of DNA bases that are installed in a constructed shape. These methods thus afford truly unique positional control on the nanoscale. Our current efforts are now centered around taking advantage of this positional control in the form of nanoscale "gadgets" for applications in the molecular biosciences.

(1) PWK Rothemund: NATURE 2006

(2) SM Douglas, H Dietz, T Liedl, B Hogberg, F Graf, W Shih: NATURE 2009

(3) H Dietz, SM Douglas, W Shih: SCIENCE 2009

BP 3.2 Mon 14:30 H43

Effect of DNA sequence variation on the dynamics of backtracking during RNA transcription — •Abigail Klopper[1,2], Justin Bois[1,2], and Stephan Grill[1,2] — [1]Max Planck Institute for the Physics of Complex Systems — [2]Max Planck Institute of Molecular Cell Biology and Genetics

The transcription of information encoded in the genome is facilitated by RNA polymerase, a macromolecular machine which steps along a DNA template, assembling and extruding a complementary RNA transcript. The process is typically marked by pausing events which have been linked to an inactive backtracked state, involving diffusive excursions of the polymerase along the template. In this state, the polymerase cannot elongate the RNA transcript, and productive synthesis only resumes once the polymerase has realigned with the RNA, effectively stepping out of the backtrack. Inability to recover can lead to cleavage of the transcript or termination of the process. We investigate the notion that sequence variation along the template influences the average time required for unassisted recovery. We perform simulations and numerical calculations using a hopping model with DNA sequence-specific transition rates. Motivated by results from single molecule experiments in which the polymerase is subject to mechanical force, we compute the sequence-averaged distribution of force-dependent pause recovery times. We show that DNA sequence variation rescales the distribution associated with a simple random walk and renders the polymerase less sensitive to the applied force.

BP 3.3 Mon 14:45 H43

Transcription of ribosomal RNA - a central task for rapid bacterial growth — •Stefan Klumpp[1] and Terence Hwa[2] — [1]Max-Planck-Institut fuer Kolloid- und Grenzflaechenforschung, 14424 Potsdam — [2]Center for Thoretical Biological Physics, UC San Diego, La Jolla CA, USA

Synthesis of ribosomes is essential for rapid cell growth and fast growing cells, from bacteria to cancer cells, devote a substantial fraction of their transcriptional activity to making ribosomal RNA (rRNA). Transcription of rRNA is typically characterized by dense traffic of RNA polymerases (RNAPs) along the rRNA genes, very different from the typical situation for mRNA-encoding genes, which have low transcription rates. As dense traffic is susceptible to traffic jams which may arise inevitably due to stochastic pausing of the polymerases, we asked whether there are specific constraints that govern transcription in a dense traffic situation. This perspective allows us to propose novel functions for termination/antitermination systems in bacterial rRNA transcription [1]. More general, the theoretical analysis of rRNA synthesis from a "traffic viewpoint" provides a unique perspective towards the physiological constraints and regulatory principles governing ribosome synthesis in bacterial and eukaryotic cells [1,2].

[1] S. Klumpp and T. Hwa PNAS 105, 18159 (2008)

[2] S. Klumpp and T. Hwa RNA Biol. 6, 392 (2009)

BP 3.4 Mon 15:00 H43

Human Telomeric Quadruplex Conformations studied by pulse EPR — Mykhailo Azarkh, Singh Vijay, Hartig Jörg, and •Drescher Malte — University of Konstanz, 78457 Konstanz, Germany

The Emmy-Noether group at the department of Physical Chemistry in Konstanz is engaged in developing and applying methods in Electron Paramagnetic Resonance (EPR) to study structure and dynamics of disordered materials.

Here we present for the first time distance measurements in quadruplex sequences based on pulsed EPR measurements (DEER) of double-nitroxide spin labeled DNA oligonucleotides. Telomeric quadruplex sequences have attracted much attention since a biological function of these unusual folds is anticipated. The human telomeric repeat is able to form structures that differ drastically in strand orientation and loop connectivity. Although it has been an important quest to decipher the physiologically relevant quadruplex topologies, the exact structures contributing to the mixtures present in potassium-rich solutions are still discussed controversially.

Our measurements demonstrate the presence of the all-parallel (so called propeller) and the all-anti-parallel (called basket) conformation in K+ solution, adding an important piece of evidence to the current debate.

15 min. break

BP 3.5 Mon 15:30 H43

Integrative investigation of DNA supercoiling under tension — •Robert Schöpflin[1], Hergen Brutzer[2], René Stehr[1], Ralf Seidel[2], and Gero Wedemann[1] — [1]University of Applied Sciences Stralsund, 18435 Stralsund, Germany — [2]Biotechnology Center Dresden, University of Technology Dresden, 01062 Dresden, Germany

Recent studies of high resolution single molecule experiments yielded detailed information of DNA supercoiling under applied tension. Here, an approach integrating experimental, numerical and analytical methods was used to understand these data. Linear DNA was investigated with magnetic tweezers under different concentrations of monovalent ions over a range of pulling forces and added supercoils. According to this we performed Monte Carlo (MC) simulations with a coarse-grained DNA model considering stretching, bending, twisting and electrostatics. The simulations reproduce well the experimentally observed behavior: A force and salt dependent abrupt buckling at the onset of the plectonemic phase is followed by a linear length decrease with added turns. The buckling transition is accompanied by an abrupt DNA length decrease depending on the ionic conditions. Beyond an overall qualitative agreement, the MC simulations reproduce quantitatively many of the experimental parameters. These include the slope and torque of the linear decrease after buckling as well as the jump size and the torque change during abrupt buckling. Moreover, we developed an analytical model for the description of DNA supercoiling. This model describes well both data from experiment and simulation when incorporating a reduced DNA charge.

BP 3.6 Mon 15:45 H43

Diffusion Based Looping Of Chromatin — •Dieter Heermann and Manfred Bohn — Universität Heidelberg, Institut für Theoretische Physik, Heidleberg, Germany

Chromatin folding inside the interphase nucleus of eukaryotic cells is done on multiple scales of length and time. Despite recent progress in understanding the folding motifs of chromatin, the higher-order folding still remains elusive. Flourescent in situ hybridization reveals a tight connection between genome folding and function as well as a folding into a confined sub-space of the nucleus. The folding state of chromatin reveals distinct differences from a compact conformation. A previously published model, the random loop (RL) model, explains the folding state by the formation of random loops, which themselves seem to be an ubiquitous motif of transcriptional regulation. However,

it remains a crucial question what mechanisms are necessary to make two chromatin regions become co-located, i.e. have them in spatial proximity.

The model presented here bridges the gap between statistical polymer models and an effective description of the dynamic process of loop formation mediated by the nuclear environment. Without assuming long- range forces or any active transport mechanisms, this model assumes that the formation of contacts or loops is done solely on the basis of random collisions. The probabilistic nature of the formation of temporary contacts mimics the effect of e.g. transcription factors in the solvent. Although only basic interactions are taken into account, this model is in agreement with recent experimental data.

BP 3.7 Mon 16:00 H43

An accurate approximation for the end-to-end distance distribution of worm-like chains of arbitrary stiffness — •Nils B Becker[1], Angelo Rosa[2], and Ralf Everaers[1] — [1]Labo de Physique and Centre Blaise Pascal de l'ENS de Lyon, Universite de Lyon, CNRS UMR 5672, Lyon, France — [2]Institute for Biocomputation and Physics of Complex Systems (BIFI), Zaragoza, Spain

The thermal conformations of semiflexible macromolecules are generically described by the worm-like chain model. To date, the model's fundamental quantity, the end-to-end distance distribution, is not known in closed form. We give an overview of the available approximations and exact limiting results for this distribution. We then combine all relevant exact limits into an explicit, generally applicable interpolation formula. The proposed expression accurately reproduces, at no computational cost, high-precision Monte-Carlo data, covering the full range from stiff to flexible chains and from looped to stretched configurations. Some applications are discussed.

BP 3.8 Mon 16:15 H43

Orientation Defined Stretching and Immobilization of DNA by AC Electrokinetics — •Venkatesh Alagarswamy Govindaraj[1], Simone Herth[1], Anke Becker[1,2], and Günter Reiss[1] — [1]Thin Films & Physics of Nanostructures, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld. — [2]Molecular Genetics, Institute for Biology III, Albert-Ludwigs-Universität Freiburg, Schänzlestr. 1, 79104 Freiburg.

DNA based single molecule studies, nano-electronics and nano-cargos require a precise placement of DNA in an orientation defined manner. Until now there is a lack of orientation defined stretching and immobilization of DNA for gaps smaller than several micrometers. However, this can be realized by designing bi-functionalized DNA with thiol at one end and (3-Aminopropyl) triethoxy silane on the other end, which specifically bind to gold and SiO2 layer after or during stretching. The electrode assembly consists of platinum as electrode material for applying the AC voltage and islands of gold and silicon dioxide fabricated at a distance of about 500-800 nm. The orientation defined stretching and covalent fixing of DNA was carried out at different frequency ranges of the applied electric field and observed after metallization of DNA by palladium ions in a Field Emission Scanning Electron Microscopy (FESEM).

BP 3.9 Mon 16:30 H43

DNA-DNA electrostatic frictional forces: magnitude and biological implications — •Andrey Cherstvy — IFF-2, FZ Jülich, Germany

We estimate theoretically the strength of DNA-DNA electrostatic frictional forces emerging upon dragging one DNA molecule over another one in a close parallel juxtaposition [1]. For ideally helical DNA duplexes, this friction occurs due to correlations in electrostatic potential near DNA surfaces. The latter originate from intrinsic helicity of DNA phosphate charges on the scale of 3.4 nm along DNA axis that produces a positive-negative charge interlocking along the DNA-DNA contact. For realistic, non-ideally helical DNAs, where electrostatic potential barriers become decorrelated due to accumulation of "sequence mismatches" in DNA structure, DNA-DNA frictional forces are strongly impeded. We calculate DNA-DNA frictional forces in both cases and describe their implications for sequence recognition of DNA duplexes that takes place in vivo upon cell division. We also discuss the possibilities of probing DNA-DNA intermolecular interactions in strongly confined DNA superhelical plies as obtained in single-molecule dual optical trap experiments.

[1] A. G. Cherstvy, J. Phys. Chem. B, 113 5350 (2009).