BP 15: DNA/RNA and related enzymes

Time: Tuesday 12:00–14:00 Location: H43

Topical Talk BP 15.1 Tue 12:00 H43

Protein diffusion on DNA — •Ralf Seidel — Institut für Molekulare Zellbiologie, Westfälische Wilhelms-Universität Münster, Germany — BIOTEC, Technische Universität Dresden, Germany

Diffusion is a major transport mechanism within living systems. Recently protein diffusion along elongated cellular structures (e.g. cytoskeletal filaments or DNA) that is often termed one-dimensional diffusion, has gained increasing interest. This is, because confinement of the diffusion path can enhance the success rate of localizing a target on the particular structure. Here we focus on a new aspect, namely that protein diffusion along DNA can be itself a central part of an enzymatic reaction. Using magnetic tweezers and fluorescence techniques we provide single-molecule observations of different enzymes on DNA: (i) a monomeric restriction enzyme that needs to turn itself on DNA to cut both strands of the helix, (ii) a restriction enzyme that uses energy from ATP hydrolysis to license fast diffusion originating from its target site and that finally triggers DNA degradation at a distant target and (iii) a helicase, i.e. an ATP-powered motor enzyme which unwinds duplex DNA, that uses diffusion on single stranded DNA to position itself in a correct orientation on its substrate. These examples suggest an important role of one-dimensional diffusion during fundamental biochemical reactions.

BP 15.2 Tue 12:30 H43

Intracellular Conformations of Human Telomeric Quadruplexes studied by Electron Paramagnetic Resonance Spectroscopy — •Malte Drescher — Fachbereich Chemie, Graduiertenschule Chemische Biologie und Zukunftskolleg, Universität Konstanz

Guanosine-rich nucleic acids fold into four-stranded structures called quadruplexes. In contrast to duplex structures, quadruplexes show a high degree of polymorphism with respect to topological features. The G-rich human telomeric repeats at the end of the chromosomes have generated much interest. As a result of their potential to switch between folded and unfolded state, the formation of quadruplex structures is suspected to play important roles in telomere maintenance and cell cycle control.

G-quadruplex topologies of the human telomeric sequence were investigated exploiting long-range distance measurements by electron paramagnetic resonance spectroscopy (EPR) in combination with site-directed spin labeling.

Using novel in-cell EPR we show for the first time that the human telomeric repeat in cellula forms a mixture of co-existing parallel and antiparallel quadruplex conformations.

BP 15.3 Tue 12:45 H43

A realistic potential for DNA-related biophysical processes — •Maria Fyta[1,2], Chia Wei Hsu[2], Greg Lakatos[2], Simone Melchionna[3,4], and Efthimios Kaxiras[2,4] — [1]Institut für Computerphysik, Universität Stuttgart, Germany — [2]Department of Physics, Harvard University, Cambridge MA 02138, U.S.A — [3]IPCF-CNR, Università La Sapienza, P.le A. Moro 2, 00185 Rome, Italy — [4]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, U.S.A.

Ab initio total-energy calculations based on density functional theory (DFT) are used to derive the coarse-grained interactions between DNA nucleotides. The interactions take into account base and sequence specificity, and are decomposed into physically distinct contributions that include hydrogen bonding, stacking interactions, backbone, and backbone-base interactions. The interaction energies of each contribution are calculated from DFT for a wide range of configurations. These interactions are then fitted by simple analytical expressions and can be used in a coarse-grained model for double-stranded DNA. Within this model each nucleotide is reduced into two sites, the base site and the sugar-phosphate site. Although this model is not derived from experimental data, it successfully reproduces the stable B-DNA structure and gives good predictions for the persistence length. It has already been used to model stretching experiments of B-DNA, as well as bubble formation within a thermalized DNA molecule. The potential may be used to realistically probe dynamics of DNA strands in various environments at the μs time scale and the μm length scale.

BP 15.4 Tue 13:00 H43

Deprotonation mechanism and the unfolding free energy landscape of a DNA i-motif — •Jens Smiatek[1] and Andreas Heuer[2] — [1]Institut für Computerphysik, Universität Stuttgart, Germany — [2]Institut für Physikalische Chemie, WWU Münster, Germany

We present the results of atomistic Molecular Dynamics simulations of a single-stranded protonated DNA i-motif. We are able to determine the full unfolding and deprotonation mechanism by using a Metadynamics approach. The release of protons which form the stabilizing hemiprotonated cytidine pairs can be identified as a two-step process which is obligatory for a partial unfolding of the i-motif into a hairpin structure. The shape of the free energy landscape indicates the native configuration as the global energetic minimum with a free energy barrier of roughly 9 kcal/mol which validates the significant stability of the i-motif in acidic solution. We further present a kinetic model for the unfolding process in good agreement to experimental results.

BP 15.5 Tue 13:15 H43

Identifying chromatin structure during DNA repair by super-resolution microscopy — •Judith Seel and Günther Dollinger — Universität der Bundeswehr München, Neubiberg, Germany

High LET irradiation of living cells using heavy ions generates a high amount of DNA DSB in close vicinity to each other. Various repair proteins and damage markers cluster to the damage sites, such as gamma-H2AX and 53BP1, forming so-called ionizing radiation induced foci of a gross size of about 1um. While structures of this size can be easily resolved using a conventional fluorescence microscope, its substructures cannot be resolved due to the diffraction limit of about 250nm in conventional fluorescence microscopy. For analyzing foci fine-structures systematically, Stimulation Emission Depletion Microscopy (STED) is utilized, which provides a lateral resolution of about 60nm fwhm.

With these improvements the microscopic images clearly indicate a fine-structure when 53BP1 is stained with two colors and the quantitative analysis proves its existence at a scale of a few hundert nm. Using the same methods with images where one color labels 53BP1 and the other gamma-H2AX, it can be shown that there is no total correlation between these two markers on a small scale.

Using these experimental and analytical methods it is possible to determine the way of clustering of one single DNA damage marker to DSB to clarify the structure of a DSB and the structure of chromatin architecture. Secondly, the comparison of two damage markers gives a deeper understanding of the interaction of repair markers and at the end maybe the possibility to decode the structure of DNA repair.

BP 15.6 Tue 13:30 H43

Entropy in DNA Double-Strand Break Detection and Signaling — •Yang Zhang, Christina Schindler, and Dieter W. Heermann — Institute for Theoretical Physics, Heidelberg University, Heidelberg, Germany

In biology, the term entropy is often understood as a measure of disorder - a restrictive interpretation that can even be misleading. Recently it has become clearer and clearer that entropy, contrary to conventional wisdom, can help to order and guide biological processes in living cells. DNA double-strand breaks (DSBs) are among the most dangerous lesions and efficient damage detection and repair is essential for organism viability. However, what remains unknown is the precise mechanism of targeting the site of damage within billions of intact nucleotides and a crowded nuclear environment, a process which is often referred to as recruitment or signaling. Here we show that the change in entropy associated with inflicting a DSB facilitates the recruitment of damage sensor proteins. By means of computational modeling we found that higher mobility and local chromatin structure accelerate protein association at DSB ends. We compared the effect of different chromatin architectures on protein dynamics and concentrations in the vicinity of DSBs, and related these results to experiments on repair in heterochromatin. Our results demonstrate how entropy contributes to a more efficient damage detection. In conclusion, we identify entropy as the physical basis for DNA double-strand break signaling.

BP 15.7 Tue 13:45 H43

Towards Darwinian Molecular Evolution in a Thermal Trap — Christof Mast[1], •Severin Schink[2], Ulrich Gerland[2], and Dieter Braun[1] — [1]Systems Biophysics, LMU Munich, Germany —

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The formation of polymers such as RNA and their replication is essential for the emergence of life. According to the RNA-world hypothesis the first polymerases were basic RNA strands of several hundred bases. Even with the help of surface catalysis and high monomer concentrations no polymerization of RNA longer than 20 bases could be demonstrated using prebiotic chemistry. Replication reactions are avoided by template inhibition and dilution. Thermal traps can overcome both problems: Temperature gradients in porous rock locally enhance the polymer concentration exponentially better for longer polymers. Since the mean polymer length depends on its local concentration, polymerization and trapping are mutually self-enhanced leading to a hyper-exponential escalation of polymer length. The theory is experimentally confirmed with sticky ended DNA. An extrapolation to the RNA world shows that a short 5 cm crack is likely to generate 100mers of RNA with micromolar concentrations even under unfavorable conditions. We experimentally show that thermals traps can drive exponential replication reactions: Convective flow drives a PCR while concurrent thermophoresis accumulates the replicated 143bp DNA and prevents the diffusion into the bulk solution. The time constant for accumulation is 92s while DNA is doubled every 50s.