data, a detailed mechanistic model of the allosteric conformational changes during DNA mismatch recognition by MutS is proposed.

1756-Pos Board B600
Working Mechanism of the Human Bloom's Syndrome Helicase
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Genome integrity is indispensable for unperturbed cell functioning. RecQ helicases play essential roles in genome maintenance. Mutations in three of the human RecQ isoforms (BLM, WRN or RECQL4) lead to severe diseases as the Bloom's, the Werner's and the Rothmund-Thomson syndromes, respectively, characterized by increased cancer predisposition and premature aging. Behind the serious genetic disorders stands the lack of repair mechanisms. BLM plays a crucial role in HR-based pathways by dissolving double Holliday-junctions and D-loops. The detailed working mechanism by which these ''roadblock remover'' functions are achieved is still unclear. We performed extensive kinetic, fluorescence spectroscopic and electrophoretic analyses to investigate the enzymatic cycle of BLM. In these studies wild-type and single tryptophan-containing BLM mutants were used. We demonstrate that BLM randomly and structure specifically binds DNA in the absence of nucleotide. ATP binds to DNA-bound BLM and induces a conformational change. ATP binding, hydrolysis and phosphate release occur rapidly and are followed by the rate limiting step of the cycle. This step is possibly a conformational change induced by DNA during translocation. BLM performs multiple ATPase cycles without dissociating from the DNA track. This results in the processive translocation activity of BLM. In contrast to other helicases (e.g. PcrA), BLM dissociates from the DNA strand at its 5′-end, thereby avoiding futile ATPase cycling. Our results emphasize the importance of investigating the basic working mechanism of different DNA helicases because these mechanisms may differ significantly. Moreover, understanding the basic working mechanism will greatly aid in understanding the complex functions of RecQ helicases.

1757-Pos Board B601
Direct Simulation Of Electron Transfer Reactions In DNA Radical Cations
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The electron transfer properties of DNA radical cations are important in DNA damage and repair processes. Fast long-range charge transfer has been demonstrated experimentally, but the subtle influences that experimental conditions as well as DNA sequences and geometries have on the details of electron transfer parameters are still poorly understood.
In this work, we employ an atomistic QM/MM approach, based on a one-electron tight binding Hamiltonian and a classical molecular mechanics forcefield, to conduct nanosecond length MD simulations of electron holes in DNA oligomers. Multiple spontaneous electron transfer events were observed in 100 ns simulations with neighbouring adenine or guanine bases. Marcus parameters of charge transfer could be extracted directly from the simulations. The reorganisation energy lambda for hopping between neighbouring bases was found to be ca. 25 kcal/mol and charge transfer rates of $4.1\times10\hat{}9\ s\hat{}-1$ for AA hopping and $1.3\times10\hat{}9\ s\hat{}-1$ for GG hopping were obtained.

1758-Pos Board B602
Studies of the Translocation Mechanism of Hepatitis C Virus NS3 Helicase with Computationally Mutant Constructs
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Hepatitis C virus (HCV) NS3 helicase unwinds double-stranded polynucleotide for HCV genome replication. Biochemical and single molecule studies have examined its enzymatic activity in depth, while the detailed translocation mechanism is still unclear. Our previous work has identified a list of hot-spot residues for its dynamic couplings and translocation by using an elastic network model (ENM). To further pinpoint key residues important for the polynucleotide movement, we used molecular dynamic (MD) simulation to study the conformational dynamics of NS3 helicase with computationally mutant constructs H293A, T324A, V432A and R461A. These mutations have been shown critical to the function of NS3 helicase by both experimental studies and ENM. We also simulated mutant constructs, T448A and P230A, which have only been predicted by ENM without experimental tests. Our results were consistent with experimental observations and suggested other important residues for polynucleotide translocation. Moreover, we have identified key hydrogen bond interactions between NS3 helicase and the polynucleotide for future experimental verification.

DNA, RNA Structure & Conformation I

1759-Pos Board B603
Elucidation of the Mechanism of an Epigenetic Switch by Single-molecule Assays
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The lambda bacteriophage epigenetic switch determines the growth lifestyle of the virus after infection of its host (*E. coli*). It is now clear that the switch consists of a ~2.3 kbp-long DNA loop mediated by the lambda repressor protein. Using tethered particle microscopy (TPM), magnetic tweezers and AFM, our laboratory has novel, direct evidence of loop formation and breakdown by the repressor, the first characterization of the thermodynamics and kinetics of the looping reaction and its dependence on DNA supercoiling and repressor non-specific binding. These *in vitro* data provide insight into the different possible nucleoprotein complexes and into the lambda repressor-mediated looping mechanism which leads to predictions for that *in vivo*. The significance of this work consists not only of the new insight into a paradigmatic epigenetic switch that governs lysogeny vs. lysis, but also the detailed mechanics of regulatory DNA loops mediated by proteins bound to multipartite operators and capable of different levels of oligomerization.

1760-Pos Board B604
Elasticity of Sub-micron DNA Molecules Studied with Axial Optical Tweezers
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Understanding the elasticity of sub-micron DNA molecules is important because many crucial biological structures and processes occur on this length scale. Using optical tweezers to manipulate DNA molecules, however, is difficult when molecules are shorter than about one micron. The reason is that conventional optical tweezers stretch molecules laterally in the focal plane of the microscope objective, a mode in which steric hindrances from the coverslip and other surface effects are substantial. To overcome the problem, we developed and calibrated an axial optical tweezers that makes this length scale accessible by stretching the molecule in the axial direction of the laser beam. By varying the laser intensity, different stretching forces were applied to the DNA molecule, and the axial position of the tethered microsphere was obtained from its diffraction pattern.
We measured the force-extension relationships of four short ds-DNA molecules, which are 1298 bp-, 662 bp-, 390 bp-, and 247 bp-long, using the axial optical tweezers. Using a modified worm-like chain (WLC) model for the extended DNA molecule that incorporates excluded-volume entropic effects from the coverslip and microsphere are taken into account, we obtained effective persistence lengths and excluded-volume forces for these molecules. The fitted values for the persistence length decrease with the contour length of the DNA, which is qualitatively consistent with observations by Seol et al. on longer, micron- and sub-micron sized constructs (Seol 2007). Moreover, the excluded-volume forces are close to the theoretical predictions by Segall et al (Segall 2006).

1761-Pos Board B605
Structure Conversion Of Human Telomeric Sequence Studied By Single-molecule Tethered Particle Motion
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Telomeres contain G-rich tandem repeats of single-stranded DNA sequences at 3′ tail. The G-rich sequences can be folded into a secondary structure named G-quadruplexes by Hoogsteen base pairing in the presence of monovalent cations (such as Na+, K+). The folding of telomeric DNA into the G-quadruplexes may inhibit telomerase activity for the proliferation of cancer cells. Moreover, the change of a quadruplex conformation may play an important role in biological effect. Thus, understanding structure conversion between the folded and unfolded G-quadruplex structures, and how the structure conversion is mediated by ions, its anti-sense sequence and its stabilizers are important to telomere biology. Here, we have directly monitored the conversion between the folded and unfolded structures in human telomeric AGGG(TTAGGG)3 sequence by the single-molecule tethered particle motion (TPM) method. TPM method monitors the DNA length change caused by the G-quadruplex formation, and allows us to monitor the conversion mechanism

of telomeric sequences in real-time. In the presence of its antisense sequence, the folded G-quadruplex structures (in 150 mM Na+) can be disrupted and converted to the unfolded conformation, and the conversion frequency depends strongly on the antisense concentrations. In the great excess of antisense sequence, the conversion efficiency is about 10 % in our single-molecule essay (N >100). However, in the presence of Li+ ions, the efficiency of antisense interaction increases significantly to 50 %. Since Li+ ions have been proposed to destabilize the G-quadruplex structure, our results suggest the antisence sequence interacts with the unfolded or, at least, partially unfolded state of telomeric sequences. Experiments of structure conversion between 150 mM Na+ and 100 mM K+ ions, and effects of structure conversion in the presence of a G-quadruplex stabilizer (BMVC) will also be discussed.

1762-Pos Board B606

Conformational Stabilization Of G-quadruplex DNA By Metalloporphyrins

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Formation and stabilization of the human telomeric G-quadruplexed DNA *in vitro* has been found to inhibit the enzyme telomerase, which is overactivated in tumor cells. Quadruplex interacting agents (QIAs), ligands that bind and stabilize the human telomeric G-quadruplex DNA, have been recognized as potential chemotherapeutic agents. Porphyrins are promising QIAs with the additional versatility of central metal-ion chelation. In the present study we have investigated the effects of various metal ions (Co (III), Cr (III), and Mn (II)) bound to the base porphyrin mesoporphyrin IX (MPIX) on the overall binding selectivity and specificity with the G-quadruplex conformation, using fluorescence, absorbance and circular dichoism spectroscopies. Our data suggests that all three metal-porphyrin derivatives exhibit high binding selectivity for G-quadruplex over double-stranded DNA, and appear to promote a conformational switch from antiparallel to parallel quadruplex conformation. Changes in the metalloporphyrin absorption spectra on binding with the G-quadruplex DNA suggests intercalating, groove binding or external stacking interactions. In addition, melting curve data reveal that all three metalloporphyin derivatives can stabilize the G-quadruplexed DNA over N-methyl mesoporphyrin IX (NMM), with a central methyl group instead of a metal ion, in the order: NMM < Co(III) MPIX < Mn(II) MPIX < Cr(III) MPIX. Consequences of our studies for design of potential new QIAs will be discussed.

1763-Pos Board B607

Simulated Single-Molecule FRET Trajectories: A Comparative Analysis Between Three Telomeric G-quadruplexes

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The human telomeric DNA sequence d[AGGG(TTAGGG)3] is known to have multiple conformations in vitro. The primary objective of this study is to understand folding and unfolding mechanisms of three human telomeric quadruplexes. Relative stabilities of two quadruplex structures, an anti-parallel and parallel form, were studied using molecular simulations and molecular modeling techniques. A third mixed form which contains the sequence d[TTGGG(TTAGGG)3A], along with the parallel and anti-parallel form was pushed away from its original conformation via a bias command on the sugar-phosphate backbone. It was found that the anti-parallel conformation was the most stable, in that it remained closest to its original conformation. Common characteristics are seen throughout the simulations, particularly stacking near the 3′ end that outlasts the rest of the structure. In an effort to understand the unfolding mechanism or transition state between observed structures, theoretical FRET signals were calculated by analyzing the movement of backbones during simulation. The movement of the backbone in simulations supports published results, namely that similar structures are seen in other studies, and the theoretical FRET signals show similarities to single molecule studies. Clustering by rmsd values shows 7 distinct possible unfolding mechanisms with similarities to published results. A more complete understanding of the stabilizing and destabilizing factors involved in quadruplexes will allow further research into the possible manipulation of the cell cycle and has been cited as an important and promising aspect in the field of cancer research.

1764-Pos Board B608

Holliday Junction Mechanics Studied Using an Angular Optical Trap

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The Holliday junction is a four-way DNA structure which plays a crucial role during homologous recombination and double-stranded DNA break repair. In vivo, the branch point can migrate spontaneously or with the assistance of helicase-like motor proteins. In this work, we mechanically migrate a Holliday junction using an angular optical trap. While applying a constant tension to the DNA molecule, we simultaneously measure the extension change and torque as the junction is torsionally driven to a new position. We determine that there exists a simple thermodynamic relationship between the torque required to stabilize the junction and the force working to destabilize it. We propose that this assay can have important uses in single molecule studies of motor proteins, particularly as a calibrated nano-torque wrench.

1765-Pos Board B609

Fullerenes May Induce Physical Changes of DNA - an Optical Tweezers Study

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Fullerenes are fascinating symmetric carbon nanostructures. Nowadays, they are widely used because of their characteristic physical and chemical properties. Until now research has mainly focused on commercial applications of fullerenes. Only a few investigations have addressed the potential biological hazards, one of which is that fullerenes are believed to alter the elastic properties of DNA upon (irreversible) binding.

In our experiments we use optical tweezers with sub-piconewton and nanometer resolution to probe the structural changes and the potential damages that fullerenes may induce on single DNA molecules. Force-extension relations of these molecules are obtained under physiological conditions while varying the concentration of different types of fullerenes, through well-defined microfluidics, in order to assess hypothesized damages. Custom-made Labview software allows for precise equipment control, various feedback options, and very fast on-the-fly data streaming.

It has been theoretically predicted [1] that certain fullerenes can function as a minor-groove binder to double-stranded DNA, thus altering its elastic properties significantly. This may be why fullerenes are capable of causing severe damage inside living organisms. They form DNA regions that are inaccessible which prevents proper enzymatic catalysis. A further goal of the study is to establish fullerenes as a tool for a more detailed investigation of DNA-minor-groove binding as well as DNA-protein interactions, such as the traffic of polymerases or the packing by prokaryotic proteins. [1] Zhao, Striolo, and Cummings: BiophysJ (89):3856-62, 2005.

1766-Pos Board B610

Kinetics of DNA force-induced melting

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Force spectroscopy studies probe nucleic acid structures by exerting tension along the molecule. As it is stretched, double-stranded DNA reveals a sudden increase in length at a constant force, a transition referred to as overstretching. Thermodynamic and chemical evidence have demonstrated that overstretching is actually force induced melting, a transition to single-stranded DNA as base pairing and base stacking are disrupted. We present a predictive model of force induced melting in which thermal fluctuations induce local melting and re-annealing of DNA. These fluctuations are stabilized by the application of tension during the overstretching transition, favoring the conversion to single stranded DNA as the applied force is increased, analogous to the thermal melting of DNA. This model quantitatively predicts small changes in the melting force as the pulling rate is varied. We then test our model for force-induced melting by systematically measuring the midpoint of the transition as a function of pulling rate. Our results suggest that DNA force-induced melting occurs cooperatively with a domain size of 100-200 base pairs.

1767-Pos Board B611

Spectroscopic Studies of Position-specific DNA 'Breathing' Fluctuations at Replication Forks and Primer-Template Junctions

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The properties of single- (ss) and double-stranded (ds) DNA sequences at replication forks and primer-template DNA junctions are central to the function of the protein complexes that drive DNA replication, transcription, recombination and repair. Significant base-pair 'breathing' (or 'fraying')