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.

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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.

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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.

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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')

occurs at these ss-dsDNA junctions and the physiological consequences of this 'breathing' is not well understood, in part because it has been difficult to measure in a sequence-specific manner in biological systems. We have used single and dimer pairs of 2-aminopurine (2-AP) residues, site-specifically placed at various positions on both sides of the ss-dsDNA junctions of DNA constructs, as spectroscopic probes of this breathing. Replacing adenosine with 2-AP in DNA has minimal biological or physical consequences, and these moieties absorb, fluoresce and display CD spectra at wavelengths >300 nm where other nucleic acid and protein components are transparent. The optical properties of these constructs have been used to measure the position-specific extent and nature of the fluctuations of forked and P/T DNA junctions. We find that spectroscopically measurable melting penetrates ~2 bps into the interior of the duplex region of these junctions under physiological conditions. In addition 2-AP bases in ssDNA loci directly adjacent to these junctions display significantly more unstacked character than do 2-AP probes located within long ssDNA sequences. Quenching of 2-AP fluorescence with acrylamide has independently confirmed these results. These local and transient DNA conformations have possible biological significance as interaction targets for DNA-manipulating enzymes, and we are using these breathing properties of DNA in isolation as a platform to study helicase activity on duplex DNA and ultimately the coupling of these helicases to the other components of the 'macromolecular machines of gene expression'.

1768-Pos Board B612

Conformational Dynamics between B- and Z-DNA probed via single-molecule FRET

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Since the first discovery of the left-handed DNA structure, **Z-DNA**, in 1979, its biological role has been under constant debate, partly because of the unclear understanding of the Z-DNA formation mechanism in the cell.

Here, we report the first single-molecule FRET experiments on Z-DNA formation. A series of DNA duplexes containing varying CG repeats and different junction numbers were tagged with Cy3 (donor) and Cy5 (acceptor) for FRET measurements, and biotinylated at the end for surface immobilization. Prism-type single-molecule FRET setup was used to monitor the conformational dynamics of hundreds of single molecules simultaneously at varying salt concentrations and with or without Z-DNA inducing protein factors.

The salt-induced B-to-Z transition occurred fast with transition time of ~ 10 seconds at 5 M of $NaClO_4$, and was reversible because B-form DNA was readily recovered when the salt concentration was reduced back. The transition due to the Z-DNA binding domain ($hZ\alpha_{ADAR1}$) from the human editing enzyme, double-stranded RNA adenosine deaminase, however, was relatively slow with ~3 minutes of transition time. Contrary to the salt-induced cases, B-form DNA was not readily recovered after protein removal, which implies the tight binding between Z-DNA and $hZ\alpha_{ADAR1}$. We determined the free energy change of protein-induced B-Z transition by measuring the equilibrium constant of duplex DNAs with varying CG repeats. The junction free energy was also determined by comparing duplex DNAs with one B-Z junction or two B-Z junctions.

1769-Pos Board B613

DNA Conformation and Biomolecular Motors: New Nanomedicine Research Targets

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DNA supercoiling is a feature of almost all DNA molecules. It is a powerful thermodynamic force that drives and directs many DNA associated processes in vivo. The level of supercoiling or DNA spatial conformation is constantly changing due to the activities of proteins and the environmental conditions of the cell. Local and temporal changes in DNA supercoiling affect many cellular processes such as replication, transcription recombination and chromosome organization.

DNA biomolecular motors such as DNA topoisomerases and DNA translocases are responsible for maintaining the steady state of supercoiling essential for cell viability. In prokaryotes, DNA supercoiling is expected to play an important role in site-specific recombination, a fundamental process to achieve resolution of dimeric chromosomes, allowing plasmids and chromosome segregation and consequently cell division. During this process, DNA undergoes multiple conformational changes due to the activity of Tyrosine recombinases and a DNA translocase known as FtsK.

I use cell biology, biochemical and biophysical techniques to study the role of DNA biomolecular motors and DNA topology in different cellular processes. In vitro, we demonstrate the topology dependence of the different steps in site-specific recombination events using DNA substrates with different superhelical density. By TIRFM, I characterize at the level of single molecule the activity of DNA molecular motors. Using high-resolution amplitude modulation atomic force microscopy (AM-AFM) in physiological buffer we characterize the nature of the forces that drive relevant DNA conformational changes by itself or after protein interaction during site-specific recombination events. Additionally, we observe for the first time the dynamics of DNA and the conformational changes of DNA during site-specific recombination events imaged by high-speed AFM at time resolutions up to 20 ms and sub-nm spatial resolution. Our current research is focus on DNA biomolecular motors as new nanomedice targets.

1770-Pos Board B614

Defect Excitation In Sharply Bent Dna And Its Micromechanical Consequences

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Kinkable defect excitation in sharply bent DNA molecules have drawn attentions in recent years. Such excitations were recently observed in molecular dynamics simulations, and were experimentally observed in circular DNAs of 64 - 65 base pairs. In addition, it was shown that rare excitation of flexible defect provided an explanation to the unusually large cyclization probability of 94 bp DNA reported recently. In this presentation, we show that defect excitation also explains a few other experiments where unusual DNA mechanical responses were observed, and we present our predictions of other measurable DNA mechanical responses for future experiments. In addition, we provide a new experimental evidence demonstrating the breakdown of the traditional WLC model when DNA is sharply bent. Finally, we discuss the molecular details of the possible defects based on our molecular dynamics simulations.

1771-Pos Board B615

Manipulating Single dsDNA Molecules To Study Force Induced Phase Transitions

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Double stranded DNA (dsDNA) exists in a variety of different conformations *in vivo*, even in the absence of force. We have used magnetic tweezers to study the overstretching transition that takes place when dsDNA is pulled at constant force. We show that during this transition there is no significant single stranded DNA formation and the relaxation to the original B-DNA form exhibits variable hysteresis depending on the pulling ends. The extent of this hysteresis is also dependent on the type of salt used decreasing with increased screening efficiency, suggesting that the overstretched state is not melted DNA. We studied the effect of ion concentration for several monovalent cations and anions. Our results are consistent with existing theory that predicted that the overstretched states are forms of dsDNA that depend on the pulling technique, where 5'5' stretching produces a narrow fiber and 3'3' stretching produces a more widely spaced ladder with each case presenting a different characteristic charge spacing. In addition we studied the shear force required to denature long dsDNA when a constant force is applied to the 3'3' ends or the 5'5' ends. For lambda phage dsDNA, the critical forces for shearing by pulling from the 5'5' and 3'3' ends are 124.4 pN and 141.3 pN respectively, whereas the overstretching force is 65 pN for both cases. Given that short dsDNA molecules that shear before they completely overstretch have the same shear force for both pulling techniques, these results further support the theoretical proposal that overstretching dsDNA by pulling from the 3'3' ends produces a different structure than pulling from the 5'5' ends.

1772-Pos Board B616

Study Of Sequence Dependent Homolog Pairing With A Single Molecule Assay

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One of the most prominent forms of shuffling genetic material is homologous recombination. Sexual reproduction, meiosis, is a hallmark of eukaryotic life on earth and is accompanied by homologous recombination of chromosomes which maximizes diversification while minimizing DNA damage. Different mechanisms have been proposed to explain how double stranded DNA (dsDNA) homologues find each other and lock together with their sequences matched so that crossovers between chromosomes result in new chromosomes;