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;