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Predicting the Mechanism and Kinetics of the Watson-Crick to Hoogsteen Base Pairing Transition

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and of tetraalkylammonium⁺ (TXA⁺) with a model hairpin oligonucleotide having the sequence GA₅C₄T₅C. As the size of the monovalent cation increases, its association with the coil conformation is enhanced and its association with the hairpin conformation is diminished. These changes can be ascribed to the hydrophobic surface of TXA⁺, increasingly attracted to the hydrophobic coil conformation and increasingly rejected by the hydrophilic hairpin conformation. Thus TXA⁺ acts as a denaturant, markedly lowering the melting temperatures of duplex DNA.

2777-Pos Board B154

Base-Pair Level Analysis of DNA Four-Way Junction Structure and Dynamics

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Immobile four-way junctions (4WJs) form a core structural motif of structured DNA assemblies. Understanding the sequence-level impact of four-way junctions on their structure, stability, and flexibility plays an important role in achieving Angstrom-level spatial control over nucleic acid nanodevices. 4WJs may exist in one of two stacked conformational isomers that are preferentially adopted when free in solution but forced into one or the other configuration in programmed multi-junction assemblies. It has been shown experimentally that the base sequences around the sites of strand crossover in these junctions have a significant impact on the conformational preferences of the two isomers.^{1,2} In particular, some sequences exhibit significant bias for one isomeric state, whereas others have equal preference between the two isomers. Here, we use explicit solvent and counterion molecular dynamics simulations to explore the base-pair level interactions that give rise to 4WJ conformational states using the classic Seeman J1 junction³ system as a point of reference. A pseudo-alchemical path approach is used to investigate how nicks placed at the site of the strand crossovers impact B-form DNA and crossover geometry and stability, and their dependence on core 4WJ sequence. Free energy calculations and analysis of the base-pair level degrees of freedom⁴ for each step along this path reveal the structural origin of the significant impact of base sequence on local structure, and suggest how these in turn impact global junction behaviour.

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2778-Pos Board B155

Measuring and Modeling the Effect of Single Mismatch on DNA Strand Displacement

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DNA strand displacement is an essential reaction in both DNA homologous recombination and DNA mismatch repair, and it is also heavily utilized in DNA-based computation and locomotion. Despite this ubiquity in science and engineering, sequence-dependent effects of displacement kinetics have not been extensively characterized. Furthermore, the bulk methods currently used make it difficult to study the underlying branch migration process. Here, we directly measured the branch migration kinetics of toehold-mediated strand displacement using single-molecule fluorescence in the presence of a single nucleotide mismatch. The apparent displacement rate varied significantly when the mismatch was introduced in the invading DNA strand. The rate generally decreased as the mismatch in the invader was encountered earlier in displacement. Our data reveal direct dissociation of the shorter DNA strand (incumbent) due to weakened base pairing during branch migration as the dominant pathway for DNA strand displacement. We show that a simple first passage model can quantitatively explain the salient features of the observed relationship. We expect our model to become a powerful tool to design DNA-based reaction schemes with broad functionality.

2779-Pos Board B156

DNA Origami Force Balance

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Commonly used techniques to study conformational changes of interacting macromolecules such as atomic force microscopy and magnetic or optical tweezers today feature spatial resolutions of single nanometers and piconewton force sensitivity [1]. One of the limitations of these methods is the need to couple the molecules under investigation to a cantilever tip or micrometer-sized beads. By constructing molecular force spectroscopy tools with the help of DNA origami [2-4] we are trying to overcome this limitation. Our

DNA origami-based force balance comprises a moveable lever on top of a base, which can exert defined forces in the regime between 1 pN and 10 pN between the lever and the base. The position of the lever can be detected with Transmission Electron Microscopy (TEM) and by Fluorescence Resonance Energy Transfer (FRET) in a dynamic fashion. Discrete and geometrically distinguishable conformations of our force balance can be observed when competing hybridizing DNA single-strands occupy their complementary counterparts on the structure on both sides of the lever. In model experiments where we tested different numbers of hybridizing strands on the two opposing ends of the lever, we observed surprisingly uniform populations of structures that were set in the "correct" conformation (~90%).

In a next step, we designed variants of the force balance with pre-stressed segments of DNA and investigated the dynamic switching of a Holliday junction under stress. In ongoing experiments we study the tension dependent bending behavior of DNA bending proteins.

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2780-Pos Board B157

The Occurrence of Plectonemes in Supercoiled DNA Depends on DNA Sequence

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The supercoiling state of chromosomal DNA needs to be precisely controlled by cells as it is important to many essential cellular processes such as DNA replication and gene expression. The extrusion of coiled loop structures termed plectonemes are one of the characteristic features of supercoiled DNA. Despite their fundamental importance, many basic properties of plectonemes are unknown mainly because of the lack of proper experimental tools to probe them. Our previous study, based on a side-pulling magnetic tweezers combined with wide-field fluorescence microscopy, revealed that DNA plectonemes are highly dynamic in nature. Here, we extend this technique to study the sequence-dependent localization of plectonemes. Moreover, we developed another single-molecule technique to probe plectonemes in DNA of which supercoiling state is carefully controlled by intercalation of small molecules. While the side-pulling magnetic tweezers technique allows precise control of force and supercoil density, this new technique provides high-throughput measurements by simultaneous visualization of many single DNA molecules in parallel. Using both techniques, we study the DNA sequence dependence of plectonemes and the influence of local structures such as bound proteins on the plectoneme formation. Analysis of the positions and sizes of the individual plectonemes reveals that plectonemes are either localized to or excluded from certain regions of DNA, depending on the nature of the sequence. Furthermore, we find that plectonemes can be pinned to local structures such as a bound protein or a local bubble formed by base pair mismatches. These first biophysics experiments on the sequence dependence on plectonemic supercoils have clear consequences for the non-homogenous distribution of supercoiling across the genome in biological cells.

(* The first two authors contributed equally)

2781-Pos Board B158

Predicting the Mechanism and Kinetics of the Watson-Crick to Hoogsteen Base Pairing Transition

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DNA duplexes predominantly contain Watson-Crick (WC) base pairs. Yet, a non-negligible number of base pairs converts to the Hoogsteen (HG) hydrogen bonding pattern, involving a 180° rotation of the purine base relative to Watson-Crick. These WC to HG conversions alter the conformation of DNA, and may play a role in several processes including recognition and replication. The transient nature of these processes hamper thorough experimental investigation. Molecular dynamics simulations can provide complementary insights to experiments at high spatial and temporal resolution. By using path sampling techniques, a framework that harvests molecular dynamics trajectories that undergo reactions of interest, we avoid long waiting times in stable states, thus focusing on the actual transition. Our results reveal that WC to HG conversion can proceed along different routes with a varying degree of exposure of the

purine. Furthermore, we computed the rate constants for this transition using transition interface sampling.

2782-Pos Board B159

Biophysical Mechanism of Sequence-Dependent Attraction between Double-Stranded DNAs and its Biological Significance

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The chromosome spends most of its lifetime in the interphase in which it is only loosely condensed and hence viewed as a dynamic polymer. The physical properties of DNA *in vitro* have been rigorously studied but its structural organization in a living cell and the role of such structure in epigenetics have only started to be explored, leaving the physical mechanism behind largely unknown. From our recent studies combining molecular dynamics simulations and single molecule experiments, we suggest that polycation-driven electrostatic inter-DNA attraction might play a crucial role in determining the overall condensed structure of DNA. We found that the C5 methyl group of thymines, which methylated cytosines also have, induces sequence-dependent attraction between double stranded DNAs. Such behavior is consistent with the large scale structure of chromosomes from Hi-C measurements and the observed compactness of hyper-methylated chromosomes. In this talk, I will present our recent efforts to test the hypothesis from the molecular to the cellular level that may reveal the physical mechanism of controlling the chromosome structure and dynamics for epigenetic gene regulation.

2783-Pos Board B160

G-Quadruplexes Recognition by Platinum Complexes Probed by Site-Directed Spin Labeling

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Guanine-rich oligonucleotides (either DNA or RNA) can adopt a unique stacked tetrad structure called the G-quadruplex. Formation of G-quadruplexes can be detected *in vivo* and is known to exert important biological functions. As such, G-quadruplexes are targets in therapeutic developments, and various classes of small molecules targeting G-quadruplexes, including metal complexes, are being investigated as potential new drugs. However, G-quadruplexes possess a very high degree of structural polymorphism, which rendering it difficult to obtain detailed information on the modes of interaction between G-quadruplexes and small molecules. Here, we report work on using the technique of site-directed spin labeling to investigate interaction between G-quadruplex and Pt(II) complexes. Using nitroxide spin labels attached to either the DNA or the targeting small molecule, we were able to reveal ligand induced conformational changes of the G-quadruplex as well as molecular details of the ligand binding mode. The results demonstrate a new strategy for investigating small molecule binding to G-quadruplexes.

2784-Pos Board B161

Computational Investigation of the Dissociative Recombination of Adenine, Guanine, Thymine, and Cytosine

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Dissociative recombination (DR) is a process during which a molecular cation captures an electron and subsequently dissociates into neutral particles. DR is one of the very efficient processes of molecular cation annihilation and of neutral particles production in dilute environment. The DR of small molecules such as H₂⁺ and H₃⁺ were thoroughly investigated during the past three decades. Recently, the cations of interest have been expanded from small inorganic molecules to macromolecules (*Phys. Chem. Chem. Phys.*, 2010, 12, 11670-11673).

On the other side, DR in dense environment had been neglected, due perhaps to the challenge that its experimental as well as theoretical investigation represent. However, there are so many important applications of DR in non-dilute environment. For example, DNA could lose an electron due to an ionizing radiation, which, at the same time, generated lots of low-energy electrons. The effects of secondary electrons on molecules of biological interests are currently a hot topic. It is now accepted that the attachment of slow electron on a neutral DNA may induce a strand break via dissociative electron attachments (see *Radiat. Res.* **157**, 227, 2002). Because these secondary electrons are slow, they could also be easily captured by the DNA⁺ which dissociative recombination may lead to a single strand and double strand breaks. As a consequence, the DNA chain break would later result in a mutation amongst the nucleotide bases.

We studied the DR of temporary DNA⁺ using the modern electronic structure program ORCA. Given the periodic structure of DNA, we were doing quantum calculations on Guanine, Adenine, Cytosine, and Thymine only. Our results indicate the existence of autoionizing states within the various nucleotide bases and support the possibility of DR to being a channel of DNA strand break.

2785-Pos Board B162

Elucidating the Role of Electrostatics in Condensed DNA Arrays

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As the sole long-range force in biological systems, electrostatic forces dominate interactions between charged biomolecules (e.g. DNA, RNA, nucleosomes, charged proteins) at all but the smallest length scales. However, our understanding of these electrostatic interactions is rudimentary at best, largely relying on mean field approximations of ions such as the Poisson-Boltzmann equation or counterion-condensation (CC) theories that have been shown to accurately predict electrostatic behavior for monovalent ions alone. We will present ion binding data on condensed DNA pellets that examines these highly-charged systems under varying conditions of ion concentration, ion valence, and packing density (utilizing crowding agents). From concordant measurements of DNA-DNA spacing, ion concentration, and osmotic pressure, we will present a comprehensive view of DNA-ion interactions under conditions of close packing and compare these data to various current electrostatic models. These data should prove vital to our understanding of the fundamental long-range forces that govern charged biomolecules.

2786-Pos Board B163

Design and Construct a Type of Fluorescently Labeled Circular DNA Molecules to Study DNA Topology and Topoisomerases by Fluorescence Resonance Energy Transfer (FRET)

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DNA topology is a tightly-regulated property of the DNA double helix that affects genomic stability and influences susceptibility to cancer and certain hereditary diseases, such as fragile X syndrome and autism. DNA topoisomerases that control DNA topology inside cells are important targets of anti-cancer drugs, such as camptothecin and doxorubicin, and antibacterial agents, such as ciprofloxacin. In a typical assay to study DNA supercoiling and topoisomerases, agarose gel electrophoresis is usually used to resolve DNA topoisomers produced in the reaction mixtures. Since gel electrophoresis is time-consuming and labor intensive, it is desirable to develop other assays, such as fluorescence-based assays, to study DNA topology and DNA topoisomerases. In this study, we developed a novel method to produce a type of fluorescently labeled circular DNA molecules in the milligram range to study DNA topology and topoisomerases by fluorescence resonance energy transfer (FRET). The newly constructed DNA molecules should provide new avenues to study DNA topology and topoisomerases in the future.

2787-Pos Board B164

The Effect of Local Melting of DNA on DNA Loop Formation

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Statistical mechanics of double-stranded DNA (dsDNA) is well described by the wormlike chain model (WLC) which assumes a harmonic bending potential. Such smooth bending potential may no longer be valid for large bending angles to form small loops (< 100 bp). Instead, DNA may rely on rare structural transitions such as local melting (opening) of base pairs to lower the energetic cost. In theory, open base pairs called bubbles can increase the looping probability of short DNA molecules by a few orders of magnitude, but a robust experimental validation of this theoretical prediction is lacking. Here, we investigated the correlation between local melting probability and looping dynamics of dsDNA using single-molecule fluorescence resonance energy transfer (FRET). We designed two groups of short DNA molecules with low and high melting probabilities around their center and measured their looping and unlooping rates in equilibrium. Our data allow rigorous tests of melttable wormlike chain (MWLC) models at short length scales for setting the lower limit for the free energy of bubble formation.