3519-Pos Board B247
Universal Behavior of DNA Escape, Drift, and Diffusion in Nanopores
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The behavior of single biopolymers under confinement is an interesting and complex problem. Confinement of DNA molecules in a nanopore is of particular interest due to the current attention given to nanopore-based DNA sequencing strategies. Here I report single-molecule measurements of the experimental escape time distributions of double-stranded DNA molecules initially threaded halfway through a solid-state nanopore and subjected to very small average forces. The escape time distributions are consistent with a one-dimensional first passage formulation, notwithstanding the geometry of the experiment and the potential role of complex molecule-liquid-pore interactions. I will discuss the dependence of the diffusion constant on molecule length and pore size, comparing the experimental observations to recent simulation results.

3520-Pos Board B248
Synchronous Optical and Electrical Measurements of Single DNA Molecules Translocating Through a Solid State Nanopore
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Nucleic acids and proteins are increasingly being analyzed using solid-state nanopores. The nanopore method relies on the measurement of transient conductance changes of the nanopores as charged biomolecules are being electrophoretically driven into it. Although electrical detection is highly appealing for the label-free analysis of biological samples, our understanding of the dynamics of charged biopolymers through such nanoconfined geometries is limited by the information captured by the electrical signal alone.
We report the development of an instrument capable of interrogating solid-state nanopore devices by simultaneous electrical and optical measurements. Instead of the conventional TEM-drilled nanopores, we used a method pioneered by our group, and fabricate nanopores in a thin SiNx membrane via dielectric breakdown in solution . In this presentation, we show how a range of odd behaviors of the ionic current can be explained with parallel fluorescence imaging of the nanopore. Our custom instrument allows us to: (i) study these anomalous events; (ii) probe the dynamics of DNA capture; (iii) monitor, for the first time, the creation process of a nanopore; and (iv) determine its location on the dielectric membrane;. A better understanding of these anomalous single-molecule events during DNA passage through solid-state nanopores is essential to increase reliability of nanopore-based sensing.

3521-Pos Board B249
Dna Denaturation-Supercoiling Transition at Thermophilic Temperatures
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Local DNA opening plays an important role in DNA metabolism and cell biology as the double-helix must be melted before the information contained within may be accessed. Cells finely tune the torsional state of their genomes to strike a balance between stability and accessibility. For example, while thermophilic organisms maintain relaxed or positively supercoiled genomes, mesophilic life forms use unique mechanisms to maintain a negatively supercoiled genome. Here, we use a single-molecule magnetic tweezers approach at high temperature to quantify the force-dependent equilibrium between DNA melting and supercoiling at temperatures populated by Thermophiles. We show that negatively supercoiled DNA denatures at 0.5 pN lower tension at thermophilic vs. mesophilic temperatures. This work demonstrates the ability to monitor DNA supercoiling at high temperature and opens the possibility to perform magnetic tweezers assays on thermophilic systems. More speculatively, the data provide a physical rationale for the evolution of negative supercoiling and suggest that the average DNA tension in vivo lies between 0.3 - 1.1 pN.

3522-Pos Board B250
In Situ Structure and Dynamics of DNA Origami Determined Through Molecular Dynamics Simulations
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The DNA origami method permits folding of long single-stranded DNA into complex three-dimensional structures with sub-nanometer precision. To ensure that such DNA origami objects behave exactly as designed, their structural, mechanical, and kinetic properties must be characterized at both macroscopic and microscopic scales. Transmission electron microscopy, atomic force microscopy and recently cryo-EM tomography have been used to characterize the properties of DNA origami objects, however their microscopic structures and dynamics have remained unknown. Here, we report the results of all-atom molecular dynamics simulations that characterized the structural and mechanical properties of DNA origami objects in unprecedented microscopic detail. When simulated in an aqueous environment, the structures of DNA origami objects depart from their idealized targets as a result of steric, electrostatic and solvent-mediated forces and approach the realistic and relaxed conformations. Whereas the global structural features of such relaxed conformations conform to the experimentally determined features, local deformations are abundant and vary in magnitude along the structures. For example, the Holliday junctions in the DNA origami structures adopt a left-handed antiparallel conformation, which differs considerably from their conformation in solution. The DNA origami structures undergo considerable temporal fluctuations on both local and global scales. Analysis of such structural fluctuations reveals the local mechanical properties of the DNA origami objects. The lattice type of the structures considerably affects global mechanics properties such as bending rigidity. Our study demonstrates the potential of all-atom molecular dynamics simulations to play a considerable role in future development of the DNA origami field by providing accurate, quantitative assessment of local and global structural and mechanical properties of DNA origami objects.

Protein-Nucleic Acid Interactions III

3523-Pos Board B251
Target Recognition and Degradation by an Adaptive Bacterial Immune System
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Prokaryotes use the CRISPR adaptive immune system to defend against invasive genetic elements. In Type I systems, short pieces of acquired DNA are transcribed into CRISPR RNAs (crRNAs) and loaded into the multi-subunit complex Cascade for target identification. The effector nuclease Cas3 is then recruited for target DNA degradation. Here we present single-particle negative stain and cryo-electron microscopy reconstructions of the RNA-guided surveillance complex from the E. coli bacterial immune system. These structures, along with biochemical studies, provide insight into the mechanisms of initial DNA recognition, effector nuclease recruitment, and target degradation, which are essential steps of CRISPR-based immunity.

3524-Pos Board B252
DNA Interrogation by the CRISPR RNA-Guided Endonuclease Cas9
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The CRISPR-associated enzyme Cas9 is an RNA-guided endonuclease that uses RNA:DNA base-pairing to target and cleave foreign DNA as part of an adaptive immune system in bacteria. Cas9 and CRISPR-derived guide RNAs (Cas9:RNA) have also been adapted for genome engineering applications in animals and plants, where nuclease-active and inactive versions are capable of targeting specific chromosomal sites for genome editing and gene regulation, respectively. Here we use single-molecule and bulk biochemical experiments to determine how Cas9:RNA interrogates DNA to find specific cleavage sites. We show that both binding and cleavage of DNA by Cas9:RNA require recognition of a short trinucleotide protospacer adjacent motif (PAM). Non-target DNA binding affinity scales with PAM density, and sequences fully complementary to the guide RNA but lacking a nearby PAM are ignored by Cas9:RNA. DNA strand separation and RNA:DNA heteroduplex formation initiate at the PAM and proceed directionally towards the distal end of the target sequence. Furthermore, PAM interactions trigger Cas9 catalytic activity. These results reveal how Cas9 employs PAM recognition to quickly identify potential target sites while scanning large DNA molecules, and to regulate double-stranded DNA scission.

3525-Pos Board B253
Structural Basis for Foreign DNA Integration in CRISPR Adaptive Immunity
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A key stage of CRISPR/Cas adaptive immunity in bacteria and archaea is the site-specific integration of short, foreign DNA sequences, called spacers, into

the host CRISPR locus. The small RNA products of these spacer sequences are used as guides by CRISPR proteins for silencing complementary viral sequences. Despite extensive research highlighting the maturation of the CRISPR RNA guides and the targeting step mediated by CRISPR ribonucleoprotein complexes, little is known about how spacer sequences are initially integrated into the host CRISPR locus. Recently developed in vivo integration assays revealed that Cas1 and Cas2 are the only CRISPR proteins required for this step. Here we show that Cas1 and Cas2 associate to form a protein complex. Using a combination of biochemical and structural approaches, we present the mechanism of spacer acquisition mediated by the Cas1-Cas2 complex.

3526-Pos Board B254

A Biophysical Study of the G-Quadruplex-Insulin Interaction

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The formation of guanine (G)-quadruplex structures in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) have potential effects on transcription of the insulin gene. Recent studies demonstrate that the ILPR G-quadruplexes can bind to insulin. The energetics of the binding between insulin and the G-quadruplexes formed by the most common ILPR repeat sequence have also been characterized in prior work. We have studied the proton transfer involved in the interaction between insulin and this DNA sequence by conducting isothermal titration calorimetry (ITC) experiments in various buffers and analyzing the observed enthalpy change. The transcriptional activity of a number of ILPR repeat sequences, including the consensus sequence, have been measured previously. Transcriptional activity for less common repeats is significantly lower than that of the consensus sequence but can be increased substantially by varying only one or two nucleotides. To determine the potential role of G-quadruplex formation and stability in regulating transcription, we have studied the second and third most common ILPR repeats as well as their variant sequences that exhibit increased transcriptional activity. ITC was used to characterize the energetics of the binding interaction between insulin and each of the four ILPR repeat sequences. The bulk thermodynamic measurements performed at various temperatures from 20 - 37 degrees Celsius provide insight into these biomolecular interactions.

3527-Pos Board B255

ADAR2: Towards a Structural and Kinetic Understanding of RNA Editing

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Adenosine Deaminases that Act on RNA (ADARs) are a small family of enzymes found in metazoans which edit pre-mRNAs by modifying the base adenosine to inosine. This editing results in translational mutations as inosine is interpreted as guanosine by translational machinery. Two editing events occur in humans on the Ionotropic Glutamate Receptor (GluR) pre-mRNA and are known to modify its ion permeability and resensitization kinetics. ADARs promiscuously edit perfectly complementary dsRNAs, however, in the context of complex secondary and tertiary structure, ADARs gain significant specificity for their substrates. Toward understanding the elements of ADARs which drive specificity in the context of a wild type substrate, we sought to generate a co-crystal structure of human ADAR2 with RNA and determine the structure of a flexible loop of the catalytic domain to ascertain its function. Crystals of GluR-B RNA and an ADAR2 truncation PP-R_2D have been produced in small scale screens, and work to produce larger crystals suitable for x-ray diffraction is ongoing. Several mutants of the ADAR2 catalytic domain (CD), S458G and R455A, have been solved. Both of these mutant structures give some insight into the previously undefined electron density of an unstructured loop located near the catalytic site. These structures remain incomplete, yet provide a better descriptor of the dynamicity of the loop. To understand the kinetic role of the loop we have replaced it with glycine and found that editing is strongly inhibited. Current work focuses on further mutations to the loop to determine its kinetic role. The results of these studies have shown that large complexes of ADAR2 and substrate mimics can be crystallized, which is fortuitous for further structure determination, and that the unstructured loop of the catalytic domain remains highly dynamic despite mutations to decrease mobility.

3528-Pos Board B256

Activation of PKR by Stem-Loop RNAs with Flanking SsRNA Tails

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Protein Kinase R (PKR) is a central component of the innate immunity antiviral pathway and is activated by double stranded RNA (dsRNA). PKR contains a C-terminal kinase domain and two tandem dsRNA binding motifs. In the accepted model for activation, binding of multiple PKR monomers to dsRNA enhances dimerization of the kinase domain. A minimum dsRNA length of 30 bp is required for binding two PKR monomers and eliciting strong enzymatic activation. However, short (15 bp) stem-loop RNAs containing flanking single stranded tails (ss-dsRNAs) are capable of activating PKR. Activation requires a 5'-triphosphate and the presence of both 5' and 3' ssRNA tails. The mechanism of PKR activation by ss-dsRNAs is not understood. Here, we have characterized the structural features of ss-dsRNAs that contribute to activation. We have designed a model ss-dsRNA PKR activator containing two single stranded tails of 15 nt and a 15 bp stem (5'-15-15-15-3') and made systematic truncations of the tail and stem regions. Analytical ultracentrifugation experiments combined with autophosphorylation assays were used to correlate RNA binding affinity with the ability to activate the kinase. Both tails are required for PKR activation; however, they can be truncated to produce either 10-15-5 or 5-15-10 without abolishing activation. Activity is retained upon reducing the stem to 10 bp but is lost upon further reduction to 5 bp. All of the ss-dsRNAs bind two PKR monomers in 75 mM NaCl. The loss of PKR activation is correlated with weaker PKR binding, consistent with a model where the autophosphorylation rates are proportional to the concentration of RNA species containing two PKR monomers. A 5'-triphosphate is required for activation. Although the binding affinity decreases upon removal of the 5'-triphosphate, the reduction is not sufficient to explain the loss of activation.

3529-Pos Board B257

Double-Strand RNA Binding Protein Profiling

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The double-strand RNA-binding proteins (DRBP), which are featured by the existence of evolutionarily conserved dsRNA-binding domain (DRBD), have been reported to function in a variety of significant cellular activities, involving RNA processing, RNA cleavage, RNA interference and anti-viral immunity pathways.

However, it still remains unclear about the molecular mechanism and dynamic properties underlying the interaction between DRBP and dsRNA. Recently single-molecule experiments have shown an ATP-independent diffusion/sliding activity of purified TRBP as well as its two homologs, PKR activator and R3D1-L, on double strand RNA molecules (*Koh et al., 2013*), indicating a potential generalizability of diffusion/sliding activity in dsRNA-binding proteins family. To test this hypothesis and to further characterize DRBPs in terms of substrate specificity, binding affinity, features of motion upon binding and multiple DRBD collaboration or redundancy, we examined in vivo expressed proteins with one or more DRBDs at single-molecule level.

In this research, DRBP genes have been cloned from Human Open Reading Frame Library and overexpressed in human A549 cells. Then we immobilized the protein products on quartz slide surface by *Single Molecule Pull Down (Jain et al. 2011)* and investigated their dynamic property upon biding various dsRNA substrates using *Protein Induced Fluorescence Enhancement* (*PIFE*) (*Hwang et al, 2011*). PIFE data from one of our candidates, TRBP, showed the intensity fluctuation of Cy3, the fluorescent label of dsRNA substrates, indicating that TRBP is sliding back and forth along dsRNA strand upon binding to it (Fig. 1). This result is highly consistent with the experimental observation *in vitro* (*Koh et al., 2013*).

1. Koh H. R *et al.* (2013) Proc. Natl. Acad. Sci USA 110:151-15.
2. Jain A *et al.* (2012) Nat Protoc 7:445-452.
3. Hwang H *et al.* (2011) Proc Natl Acad Sci USA 108:
7414-7418.

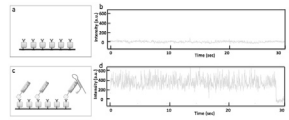

Figure 1. PIFE of TRBP bidirectional sliding along dsRNA. dsRNA concentration was 0.5nM for both control (a and b) and test (c and d). Control group was recorded without TRBP.

3530-Pos Board B258

Thermodynamic and Structural Studies of Pdx1 Binding to Elements from Natural Promoters and Near-Consensus Sites

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The production of insulin and islet amyloid polypeptide (IAPP) is limited to β-cells due to restricted expression of a set of tissue-specific transcription factors, the most well known of which is Pdx1. Naturally, insulin is expressed in much higher levels than IAPP, perhaps owing to the affinity of Pdx1 to its target sites. Like many transcription factors, Pdx1 contains regions of disorder whose function in transcription has not been adequately studied. It is well documented that the Pdx1 homeodomain binds to a core DNA recognition sequence containing the tetranucleotide TAAT, for which its consensus binding site is reported as 5′-CTCTAAT(T/G)AG-3′. Interestingly, while Pdx1 regulatory elements in the human *insulin* promoter