**3519-Pos Board B247**
**Universal Behavior of DNA Escape, Drift, and Diffusion in Nanopores**
**David P. Hoogerheide**[1,2], Jene A. Golovchenko[3].
[1]NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD, USA, [2]National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA, [3]Department of Physics, Harvard University, Cambridge, MA, USA.
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**
**Jose A. Bustamante**[1], Nick Yelle[2], Tabard-Cossa Vincent[2].
[1]University of Ottawa, Ottawa, ON, Canada, [2]Department of Physics, University of Ottawa, Ottawa, ON, Canada.
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**
**Eric Galburt**, Eric Tomko, Tom Stump, Ana Ruiz Manzano.
Washington University in Saint Louis, St. Louis, MO, USA.
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**
**Jejoong Yoo**, Aleksei Aksimentiev.
University of Illinois at Urbana-Champaign, CHAMPAIGN, IL, USA.
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**
**Megan L. Hochstrasser**, David W. Taylor, Prashant Bhat, Chantal K. Guegler, Jennifer A. Doudna.
MCB, UC Berkeley, Berkeley, CA, USA.
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**
**Samuel H. Sternberg**[1], Sy Redding[2], Martin Jinek[3], Eric C. Greene[4], Jennifer A. Doudna[1].
[1]Chemistry, University of California, Berkeley, Berkeley, CA, USA, [2]Chemistry, Columbia University, New York, NY, USA, [3]Biochemistry, University of Zürich, Zürich, Switzerland, [4]Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA.
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**
**James K. Nunez**[1], Jennifer A. Doudna[2].
[1]Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA, [2]HHMI, Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA.
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