domain. Models of the unbound p21RE, which were selected using the measured distances from a large pool generated by Monte-Carlo simulations, reveal major conformational changes at the half-site interface as compared to the bound DNA reported in a crystal structure. These results shed light on the mechanism of DNA recognition by p53.

1014-Plat
Visualizing DNA Disassociation from the Nucleosome Core Particle
Joshua Tokuda[1], Traci Topping[2], Lisa Gloss[2], Lois Pollack[1].
[1]Cornell University, Ithaca, NY, USA, [2]Washington State University, Pullman, WA, USA.
The organization of DNA in nucleosome core particles (NCP) is understood to play crucial regulatory roles in transcription, replication, recombination and repair. High resolution crystal structures deliver detailed snapshots of the NCP in its most stable conformations and reveal thousands of electrostatic interactions that mediate the stability of NCPs. However, many of the techniques that have been applied thus far are ill-suited for directly monitoring the intermediary and dissociated conformations of the NCP. To this end, we applied small angle X-ray scattering (SAXS) - a powerful technique for delivering low resolution structural details of biological molecules. Since SAXS studies of protein-nucleic acid complexes are complicated by the differences in scattering lengths between proteins and nucleic acids, we applied a contrast variation approach where we matched the solvent and protein contrasts to effectively probe the DNA component of the NCP alone. We systematically modulated the electrostatic interactions by adjusting the salt concentration of the solvent and visualized the conformational transition of the DNA from a bound to unbound state during NCP disassembly.

1015-Plat
Single Molecule Observation of Interaction between Argonaute-Guide and Target RNA and its Implication on Gene Silencing
Seung-Ryoung Jung[1], Eunji Kim[2], Wonseok Hwang[1], Ji-Joon Song[2], Sungchul Hohng[1].
[1]Seoul National University, Seoul, Korea, Republic of, [2]KAIST, Daejeon, Korea, Republic of.
Argonaute (Ago) is the catalytic core of small RNA-based gene regulation. Despite a plenty of mechanistic studies on Ago, the dynamical aspects and the mechanistic determinants of target binding and dissociation of Ago-guide remain unclear. Here, by using single-molecule fluorescence resonance transfer (FRET) assays and *Thermus thermophilus* Ago, we reveal that Ago-guide dynamically associates and dissociates with a target, and that different regions of guide-target base pairing are responsible for different steps of target recognition by Ago-guide. In addition, we show that the 3'-end of the guide strand dynamically anchors at and releases from the PAZ domain of Ago, and that the 3'-end anchoring of the guide strand greatly accelerates the target dissociation by destabilizing the guide-target duplex. Collectively, our results implicate that the target binding/dissociation of Ago-guide is executed through the dynamic interplays among Ago, guide, and target.

1016-Plat
Nucleic Acid Chaperone Activity Transforms the Energy Landscape of HIV-1 TAR RNA
Micah J. McCauley[1], Ioulia Rouzina[2], Kelly Hoadley[2], Robert J. Gorelick[3], Karin Musier-Forsyth[4], Mark C. Williams[1].
[1]Northeastern University, Boston, MA, USA, [2]University of Minnesota, Minneapolis, MN, USA, [3]Frederick National Laboratory for Cancer Research, Frederick, MD, USA, [4]The Ohio State University, Columbus, OH, USA.
Retroviral nucleocapsid (NC) proteins are nucleic acid chaperones that play a key role in the viral life cycle, including reverse transcription, where NC destabilizes the transactivation response RNA (TAR RNA) hairpin. To quantify the interaction of HIV-1 NC and TAR RNA, we use optical tweezers to exert tension upon the free ends of an individual TAR hairpin, forcing the hairpin open and then allowing it to close. We combine force-ramp experiments with a new analytical technique that quantitatively characterizes the energy landscape of equilibrium and non-equilibrium TAR hairpin unfolding and folding. We use the pulling rate dependence of the unfolding force to determine the distance to the transition state for this complex. This measurement reveals that to cause complete TAR unfolding, it is sufficient to unzip 10 bp of the 24 bp TAR hairpin stem in the absence of NC but only 5 bp of 24 in the presence of NC. Extrapolation of the measured TAR opening rate to zero pulling rate yields nearly 1000-fold faster opening with NC, equivalent to a decrease in the TAR opening barrier of 4.2 +/- 0.2 kcal/mol. Furthermore, equilibrium and complementary non-equilibrium TAR unfolding measurements using Crooks fluctuation theorem show that NC lowers the overall free energy of hairpin opening by 8.5 +/- 0.3 kcal/mol. These results demonstrate that NC destabilizes every nucleic acid base pair by strongly facilitating nucleic acid opening. This work was funded in part by Federal Funds from NCI, NIH under contract HHSN261200800001E (RJG).

1017-Plat
Below-Equilibrium DNA Topology Simplification by Type II Topoisomerases can be Explained by DNA Linking Number-Dependent Binding Affinity
Tamara R. Litwin, Susanta S. Sarkar, Ashley H. Hardin, Keir C. Neuman.
National Heart, Lung, and Blood Institute, Bethesda, MD, USA.
Topoisomerases modify and regulate the topology of cellular DNA and are essential for successful cell division and other DNA metabolism processes. Type I topoisomerases are ATP-independent and cut one strand of DNA in order to relax supercoils. Type II topoisomerases are ATP-dependent and pass a duplex DNA segment through a transient double-stranded cut in a second DNA segment to change levels of supercoils, knots, or catenanes. Whereas type I topoisomerases simplify DNA topology towards thermal equilibrium, type II enzymes can simplify DNA topology to levels below thermal equilibrium. This obeys thermodynamic laws because ATP is hydrolyzed in the process, but the mechanism by which type II topoisomerases acting on the nanometer scale are able to detect and simplify the global topology of DNA on a much larger scale is not yet known. We propose and test a model to explain non-equilibrium topology simplification by type II topoisomerases in which the enzyme affinity increases in proportion with the DNA linking number. E. Coli Topo IV has a higher binding affinity for negatively supercoiled DNA than for relaxed DNA, but the general relationship between linking number and affinity has not been established for type II topoisomerases. To determine the linking number dependent-binding affinity and to test the validity of the proposed model, we developed a method to measure the relative biding affinity of type II topoisomerases to different DNA topoisomers. The results of these measurements, in conjunction with computer simulations, permit a sensitive test of the proposed non-equilibrium topology simplification model.

1018-Plat
Single-Molecule Observation of Viral DNA Targeting by CRISPR/Cas Immune Systems
Samuel H. Sternberg[1], Sy Redding[2], Prashant Bhat[1], Martin Jinek[1], Blake Wiedenheft[3], Jennifer A. Doudna[1], Eric C. Greene[2].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Columbia University, New York, NY, USA, [3]Montana State University, Bozeman, MT, USA.
Bacteria and archaea maintain a history of viral infections by integrating small fragments of foreign DNA into specialized genomic loci called clustered regularly interspaced short palindromic repeats (CRISPRs). An adaptive immune response is triggered during subsequent infections by CRISPR-derived RNAs (crRNAs), which function together with CRISPR-associated (Cas) proteins to identify and destroy complementary viral DNA sequences. DNA targeting by crRNA/Cas ribonucleoprotein surveillance complexes proceeds via RNA-DNA base-pairing interactions and requires melting of the double-stranded substrate, as well as protein-mediated recognition of a proximal DNA sequence motif to discriminate self from non-self. How these complexes find short target sequences within the larger context of genomic DNA is unknown, and prior studies have been limited by their use of oligonucleotide substrates and dependence on bulk electrophoretic mobility shift assays that obscure all dynamics of the search process. To gain deeper insights into this critical step of CRISPR/Cas based immunity, we are using fluorescence microscopy to visualize single crRNA/Cas complexes on nanofabricated curtains of viral DNA in real-time. These experiments enable direct observation of the kinetics and position-dependence of DNA binding, and have revealed the mechanism by which phylogenetically distinct surveillance complexes use sequence information in the crRNA to locate complementary DNA targets. Ongoing work is aimed at understanding how this recognition event promotes destruction of viral DNA by dedicated Cas nucleases.

1019-Plat
Cooperative DNA-Binding Effect through DNA Allostery
yujie sun[1], Sangjin Kim[2], Erik Broströmer[3], Dong Xing[1], Jianshi Jin[1], Shasha Chong[3], Hao Ge[1], Siyuan Wang[3], Xiao-dong Su[1], X. Sunney Xie[3].
[1]Peking University, Beijing, China, [2]Yale University, New Haven, CT, USA, [3]Harvard University, Cambridge, MA, USA.
Allostery is well-documented for proteins but less recognized for DNA-protein interactions, in which DNA has been often considered as a mere template