1403-Pos
Probing Protein Diffusion and Dissociation Mechanisms on DNA Using Fluorescence-Force Spectroscopy
Ruobo Zhou[1], Rahul Roy[1], Alexander G. Kozlov[2], Timothy M. Lohman[2], Taekjip Ha[1].
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Washington University School of Medicine, St. Louis, MO, USA.
Single-stranded (ss)DNA binding (SSB) proteins tightly bind to ssDNA and protect it from degradation during DNA replication, recombination and repair. For subsequent DNA processing, SSB proteins need to be displaced from ssDNA and replaced by other proteins. The recently discovered activity that E. coli SSB can diffuse on ssDNA [1] may facilitate these processes, but little is known about the diffusion mechanism. Here we use single-molecule fluorescence-force spectroscopy [2] to study DNA-protein interactions and show that ssDNA can be progressively unraveled from the surface of a single E. coli SSB tetramer with increasing force between 1-6 pN, followed by SSB dissociation at about 9 pN. Our data also indicate that SSB diffuses on ssDNA primarily via a reptation rather than a rolling mechanism. These approaches provide unique insights into the mechanical regulation of DNA-SSB interactions and are generally applicable to many other protein-nucleic acid systems.
[1] R. Roy, Kozlov, A. G., Lohman, T. M. and T. Ha. SSB protein diffusion on single-stranded DNA stimulates RecA filament formation. Nature (2009, in press)
[2] S. Hohng, R. Zhou, M. K. Nahas, J. Yu, K. Schulten, D. M. J. Lilley and T. Ha. Fluorescence-force spectroscopy maps two-dimensional reaction landscape of the Holliday junction. Science 318, 279-283 (2007).

1404-Pos
Understanding DNA Condensation: From Simple Ions to Complex Proteins
Jason DeRouchey, Don C. Rau, Adrian Parsegian.
NIH, Bethesda, MD, USA.
We have used osmotic stress coupled with X-ray scattering to probe the thermodynamic forces between DNA helices in the presence of various cations. First, lysine, arginine, & alkylamines were investigated as a function of length. For all three systems, intermolecular forces are repulsive for mono- and divalent cations. Longer polyvalent cations mediate attractive forces and DNA spontaneously condenses into a hexagonal array. Repulsions were found to be unique for a given chemistry and independent of length, DNA-DNA attractions vary monotonically with length or charge. The magnitude of attractions increases with increasing polycation length, reaching a limiting value at 6-10 repeats. Interestingly, this limit seems to be known in naturally occurring peptides that interact with nucleic acids and utilize similarly sized repeat motifs such as the hexaarginine repeat commonly found in protamines used for DNA packaging in spermatids. To better understand the nature of complex proteins on DNA, the effect of uncharged amino acids were studied using model hexaarginine peptides. Amino acid chemistry, position and length are examined. Better understanding the cation mediated attractions and repulsions helps us to elucidate the interplay between ion entropy and the correlations that are common to nearly all theories for counter-ion induced attractions.

1405-Pos
DNA Interaction Kinetics of HIV-1 Nucleocapsid and Gag Proteins
Jialin Li[1], Christopher Jones[2], Siddhartha A. Datta[3], Alan Rein[3], Robert J. Gorelick[4], Ioulia Rouzina[5], Karin Musier-Forsyth[2], Mark C. Williams[1].
[1]Northeastern University, Boston, MA, USA, [2]The Ohio State University, Columbus, OH, USA, [3]HIV Drug Resistance Program, Frederick, MD, USA, [4]AIDS and Cancer Virus Program, Frederick, MD, USA, [5]University of Minnesota, Minneapolis, MN, USA.
The human immunodeficiency virus type 1 (HIV-1) Gag protein is essential for retroviral assembly. During viral maturation, Gag is processed to form matrix, capsid, and nucleocapsid (NC). NC is initially cleaved into the larger NCp15, then to NCp9, and finally to NCp7. NCp7 functions as a nucleic acid chaperone during retroviral replication, in which it rearranges nucleic acids to facilitate reverse transcription and recombination. The role of Gag and intermediate forms of NC in facilitating nucleic acid remodeling is not well understood, although it is likely that they also function as chaperones during viral assembly and early steps of reverse transcription. To investigate the capability of Gag and precursor forms of NC to act as nucleic acid chaperones, we use single DNA molecule stretching to probe how these proteins alter DNA aggregation, duplex destabilization, and DNA interaction kinetics. These characteristics are critical for efficient nucleic acid chaperone activity. Duplex annealing in the presence of NCp7 indicates that this protein dissociates rapidly from single-stranded DNA. In contrast, Gag inhibits DNA annealing, as indicated by strong hysteresis observed when stretching and relaxing DNA in the presence of Gag. We use a new method to measure the rate at which DNA that has been melted by force is able to reanneal in the presence of Gag and NC proteins. The results show that DNA annealing in the presence of Gag occurs on the time scale of minutes, compared to ~1 second for annealing in the presence of NCp7. Further studies of reannealing kinetics in the presence of NCp9, NCp15, and Gag deletion constructs will elucidate the role of specific protein domains in determining Gag- and NC-DNA interaction kinetics.

1406-Pos
Replacement of a Single Aromatic Residue in HIV-1 Nucleocapsid Protein Strongly Alters its Nucleic Acid Chaperone Activity
Hao Wu[1], Micah J. McCauley[1], Robert J. Gorelick[2], Ioulia Rouzina[3], Karin Musier-Forsyth[4], Mark C. Williams[1].
[1]Northeastern University, Boston, MA, USA, [2]AIDS and Cancer Virus Program, SAIC-Frederick, Inc, Frederick, MD, USA, [3]University of Minnesota, Minneapolis, MN, USA, [4]Ohio State University, Columbus, OH, USA.
The human immunodeficiency virus type 1 (HIV-1) nucleocapsid (NC) protein plays an essential role in several stages of HIV-1 replication. One important function of HIV-1 NC is to act as a nucleic acid chaperone, in which the protein facilitates nucleic acid rearrangements important for reverse transcription and recombination. NC contains only 55 amino acids, with 15 basic residues and two zinc fingers, each having a single aromatic residue (F16 and W37). Despite its simple structure, HIV-1 NC appears to have optimal chaperone activity, including the ability to strongly aggregate nucleic acids, destabilize nucleic acid secondary structure, and facilitate rapid protein-nucleic acid interaction kinetics. Here we use single molecule DNA stretching experiments to measure the characteristics of wild type and mutant HIV-1 NC that are important for nucleic acid chaperone activity. This work allows us to directly relate HIV-1 NC structure with its function as a nucleic acid chaperone. By stretching single DNA molecules in the presence of these proteins, we measure the ability of the proteins to destabilize dsDNA, and when the protein is relaxed we determine the capability of the protein to facilitate nucleic acid annealing. We show that the single amino acid substitution W37A significantly slows down NC's DNA interaction kinetics, while retaining NC's helix-destabilization capabilities. In contrast, the substitution F16W results in a protein that strongly resembles wild type NC. Thus, elimination of a single aromatic residue strongly reduces NC's chaperone activity. The results of these studies are consistent with in vivo HIV-1 replication measurements, which show that the aromatic W37 residue is required for efficient retroviral replication.

1407-Pos
Correlation Between DNA Binding Thermodynamics and Functional Behavior of Pol I DNA Polymerases
Hiromi S. Brown, Vince J. LiCata.
Louisiana State University, Baton Rouge, LA, USA.
Primer-template DNA (pt-DNA) binding by DNA polymerases is the first step of the polymerization cycle. We have previously characterized the thermodynamics of pt-DNA binding with respect to temperature for the ''large fragments'' of DNA polymerase I, Klentaq and Klenow, from *Thermus aquaticus* and *Escherichia coli*, respectively. Results with both polymerases showed that DNA binding is enthalpy-driven near their respective physiological temperatures. Here, nucleotide incorporation activity was measured with respect to temperature to examine how the thermodynamics of initial pt-DNA binding relates to the enzymatic activities of Klentaq and Klenow. For both polymerases it is observed that a negative enthalpy of initial binding (ΔH) is required for nucleotide incorporation activity, and that a negative entropy of binding ($T\Delta S$) inhibits the catalytic activity. Nucleotide incorporation activity was also examined with respect to KCl concentration. As reported previously, thermodynamic linkage plots for pt-DNA binding with respect to KCl concentration ($\partial \ln 1/Kd$ versus$\partial \ln[KCl]$) exhibit negative slopes for both polymerases and indicate net ion releases of ~3 and ~5 ions upon pt-DNA binding by Klentaq and Klenow, respectively. Interestingly, linkage plots for the steady-state rate of incorporation activity with respect to KCl concentration ($\partial \ln 1/ssRate$versus$\partial \ln[KCl]$) exhibit the same slopes as the linkage plots of pt-DNA binding. This result suggests that salt dependence of initial pt-DNA binding dictates the salt dependence of the overall incorporation activity. It is striking that for both salt and temperature dependences, the detailed thermodynamics of DNA binding so directly correlate with overall functional behavior.
This work is supported by National Science Foundation.

1408-Pos
Dna Binding and Translocation by S. Cerevisiae RSC
Allen Eastlund, Shuja Malik, Christopher Fischer.
University of Kansas, Lawrence, KS, USA.
We have studied the mechanisms of double-stranded DNA binding and double-stranded DNA translocation by a truncated construct of the RSC chromatin