1403-Pos
Probing Protein Diffusion and Dissociation Mechanisms on DNA Using Fluorescence-Force Spectroscopy
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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
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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
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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
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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
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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
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We have studied the mechanisms of double-stranded DNA binding and double-stranded DNA translocation by a truncated construct of the RSC chromatin

remodeling complex from S. cerevisiae. We monitored the double-stranded DNA translocation activity of RSC through its ability to actively displace streptavidin from biotin labeled double-stranded DNA. This displacement activity is ATP-dependent and is correlated with the translocation of the complex along the double-stranded DNA. We have also characterized the double-stranded DNA translocation of RSC through the use of a fluorescence-based stopped-flow assay in which the translocation of the complex is monitored through the interactions of the complex with fluorophores attached to the ends of the DNA. The kinetics of double-stranded DNA binding were also monitored using a fluorescence-based stopped-flow assay. The results indicate that double-stranded DNA binding occurs through a two-step mechanism, similar to what has been reported for other chromatin remodeling complexes and genetically related helicases.

1409-Pos

***E.coli* SSB Under Tension**

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E. coli SSBs (single-stranded DNA binding proteins) are essential accessory proteins in replication, recombination and during DNA repair. They not only protect ssDNA from chemical and nucleolytic attack, but also associate with many genome maintenance proteins. *E. coli* SSBs are tetrameric and have at least two binding modes: SSB_{35} and SSB_{65}, which bind 35 and 65 nucleotides, respectively. Proteins in the SSB_{35} mode bind cooperatively, interacting with each other and creating long nucleoprotein filaments. In this study, we present mechanical studies of SSB bound to ssDNA using dual trap, high-resolution optical tweezers. This assay allows us to probe the interaction of SSBs to ssDNA constructs of various lengths, in real time, with nanometer resolution. By directly detecting wrapping of ssDNA by a single protein, we are able to characterize the thermodynamics and kinetics of nucleoprotein complex formation. Mechanical pulling of our constructs in the presence of SSBs reveals that the protein condenses ssDNA in the force range 0-8pN and that tension can be used to modulate the binding mode of SSBs. Binding kinetics further indicate that SSBs bind to ssDNA in two successive processes consisting of a loose binding step in which the protein associates weakly with its substrate, followed by a wrapping step in which ssDNA is condensed.

1410-Pos

Analysis of Nucleic Acid Conformations and Amino Acid Propensities in Single-Stranded Binding Proteins

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Single stranded DNA- or RNA-protein complexes appear in a number of biological pathways, particularly those relating to DNA replication, gene regulation, and chromosome stability. Protein-nucleic acid binding in these complexes has been studied for some time, and a number of structures of these complexes exist; however, few consistent rules for binding have been determined. In particular, DNA and RNA conformation appears to vary widely, even when bound to homologous proteins (e.g., telomere end-binding proteins). No systematic study of nucleic acid conformational has been done, so it is unknown how different these conformations actually are from each other or from double-stranded DNA and RNA. In addition, no comparison of amino acid propensity for single stranded versus double stranded nucleic acids has been done. To those ends, we have built a non-redundant database of single stranded binding proteins. We have calculated various base step conformational and find wide statistical distributions for these parameters, but with some significant differences from double stranded nucleic acids.

1411-Pos

Binding and Bending Parameters of Integration Host Factor to Four-Way Holliday Junction

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Binding and Bending Parameters of Integration Host Factor to Four-Way Holliday Junction

Integration host factor (IHF) is a small heterodimeric protein that sequence specifically binds the minor groove of DNA and facilitates a bend of nearly 180 degrees. This bending is crucial for cellular processes such as recombination, replication and transcription. Previous work in the Mukerji lab characterized the binding properties of the structurally similar but sequence non-specific DNA-binding protein HU, to duplex and Holliday junction DNA. This research demonstrated that HU binds to the central region of the junction with nanomolar affinity and prefers the stacked form of the Holliday junction. Given the similarities in structure and function of HU and IHF, we elected to study the binding capability of IHF to the Holliday junction. We have compared the binding affinity of IHF for Holliday junction DNA with its binding affinity to duplex DNA with and without a consensus sequence. Binding measurements were performed using florescence intensity and anisotropy methods and confirmed with the gel mobility shift assay. All binding assays established that IHF binds to the Holliday junction lacking a consensus sequence with high affinity (~3 nanomolar Kd) similar to HU, suggesting that both proteins might recognize and bind similar structural aspects of the Holliday junction. Moreover, anisotropy affinity measurements demonstrated that IHF binds the Holliday junction with similar affinity as it does to duplex DNA containing its consensus sequence. The span of binding capabilities exhibited by IHF indicate that it can function both as a specific and non-sequence specific DNA binding protein.

1412-Pos

Inhibition of Eukaryotic RNase P

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Ribonuclease P (RNase P) is an essential RNA enzyme found in all phylogenetic domains that is best known for catalyzing the 5' endonucleolytic cleavage of precursor transfer RNAs (pre-tRNAs). In bacteria, the enzyme consists of a single, catalytic RNA subunit and one small protein, while the archaeal and eukaryotic enzymes have 4-10 proteins in addition to a similar RNA subunit. The RNA has been shown to act as a ribozyme at high salt *in vitro*; however the added protein optimizes kinetics and makes specific contacts with the pre-tRNA substrate. The bacterial protein subunit also appears to be required for the processing of non-tRNA substrates by broadening substrate recognition tolerance. In addition, the immense increase in protein content in the eukaryotic enzyme suggests substantially enlarged capacity for recognition of additional substrates. Recently, intron-encoded box C/D snoRNAs and HRA1 RNA were shown to be likely substrates for yeast RNase P. In addition, yeast RNase P seems to be inhibited by single stranded RNA. This inhibition was shown to be specific to *S.cerevisiae* RNase P but not bacterial RNase P with poly-ribonucleic acid homopolymers having varying levels of inhibition (polyG>polyU>>poly A>>>polyC). This inhibition is also size dependent with larger RNA being inhibitory. In addition, the sequence specificity seen with the small homoribopolymers is lost, as larger RNA of various sequences show relatively high levels of inhibition. To further characterize the extent of RNase P inhibition by these various inhibitors, the location of the inhibitor contact is being mapped by crosslinking. Individually 6xHis tagged strains are being used to enable isolation of crosslinks induced by UV light. The characterization of the eukaryotic specific inhibitor interaction with yeast RNase P will provide further information about the evolution of the essential activity of tRNA processing.

1413-Pos

Finding the Right 'Mis'Match: Millisecond Conformational Dynamics of MutS-DNA Complex During DNA Damage Recognition

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Errors in replication and recombination cause base-pair mismatches and insertion-deletion loops (IDLs) in DNA, which, if unchecked, lead to mutations implicated in cancer and heart disease. Thus, repair pathways that detect such errors and initiate their repair are vital to preserving DNA integrity. This study focuses on prokaryotic *T. aquaticus* (*Taq*) MutS and its eukaryotic homolog *S. cerevisiae* $MutS_{\alpha}$, both proteins that recognize mispairs and IDLs in DNA and recruit downstream proteins to rectify the damage. Structural studies have shown that MutS-bound DNA is bent at the mispair/IDL site. However, the underlying mechanism of recognition of these target sites is not well understood. In particular, the role of DNA flexibility at the target site in the recognition mechanism remains unclear. We employ nanosecond laser temperature jump (T-jump) to perturb the MutS-DNA complex, and monitor the conformational relaxation dynamics on microsecond-to-milliseconds time-scales relevant to the formation of the initial recognition complex that triggers DNA repair. We have carried out equilibrium and kinetic measurements on DNA labeled with 2-aminopurine (2AP) adjoining a T-bulge, in complex with *Taq* MutS and$MutS_{\alpha}$. Equilibrium fluorescence measurements as a function of increasing temperature reveal a sharp increase in 2AP fluorescence at ~40°C for $MutS_{\alpha}$, and at ~70°C for *Taq* MutS, indicating a significant conformational change in the MutS-DNA complex near their optimal physiological temperatures. Relaxation kinetics monitored with 2AP fluorescence in response to a rapid T-jump show relaxation kinetics in *Taq* MutS near 70°C, occurring with a time constant of a ~10 milliseconds. This rapid phase has not been observed in previous dynamics studies, and we propose that it may correspond to the transition from a non-specific MutS-DNA complex to a specific complex that signals initiation of repair.