**2146-Pos Board B116**
**Single-molecule Studies Of p53 Sliding Along DNA**
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To locate its target site on DNA, a transcription factor (TF) must recognize its site amongst millions to billions of alternative sites on DNA. Studies suggested that TFs in order to facilitate their search process alternate between 3D diffusion in solution and 1D diffusion along DNA. The duration of such a search depends on the rate at which a TF slides along DNA and the frequency with which it alternates between 1D and 3D diffusion.
We are interested in the 1D searching mechanism of p53, a transcription factor that functions as a tumor suppressor in human cells. We are using single-molecule techniques to observe diffusion of the fluorescently labeled p53 proteins along individual, stretched DNA molecules. In our previous studies, we determined the 1D diffusion coefficient of p53 protein. By measuring the 1D diffusion of the p53 protein as a function of ionic strength, we determined that the p53 protein maintains close contact with the DNA duplex and tracks the helical pitch. Current work involves the characterization of the role of the different protein domains in sliding. The C-terminus of p53 is suggested to be responsible for keeping the protein in contact with DNA by non-specifically interacting with the negatively charged backbone of DNA, while the core domain is suggested to be responsible for specifically binding the target site. We will present single-molecule data on the diffusional mobility along DNA of the C-terminal domain of p53, the p53 lacking its C-terminus, and the core domain of p53.

**2147-Pos Board B117**
**Single-Molecule Observation of the Rotational and Translational Movement of the PCNA Sliding Clamp Along DNA**
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Using single-molecule TIR (total internal reflection) fluorescence microscopy we study the dynamics of the eukaryotic sliding clamp PCNA (proliferating cell nuclear antigen). PCNA is a homotrimeric ring that plays multiple roles at the replication fork as a processivity factor for polymerases and as a molecular tool belt tethering a variety of nucleic-acid enzymes to the DNA. First, we studied the 1-dimensional diffusion of PCNA loaded around a well-stretched and doubly-tethered λ-DNA molecule. We found that the diffusion coefficient of PCNA does not vary with ionic strength suggesting that PCNA maintains electrostatic contact with DNA as it slides. Further, we found that the diffusion coefficient of PCNA is relatively insensitive to changes in viscosity when high molecular viscogens are used. This observation suggests that PCNA tracks the DNA double helix as it slides. However, increasing the hydrodynamic radius of PCNA by coupling the protein to QDot, resulted in a diffusion coefficient that was over an order of magnitude higher than expected for a helically tracking protein. We therefore propose that PCNA uses both helically tracking and non-helically tracking modes of diffusion and speculate why this may be advantageous for the many roles played by PCNA. Finally, we seek to extend our dynamic studies of PCNA from naked DNA to the context of a replication fork. Towards that end, we are using *Xenopus laevis* egg extracts in combination with single-molecule fluorescence imaging to visualize individual PCNA trimers during polymerase-mediated DNA synthesis and eventually during DNA replication.

**2148-Pos Board B118**
**Linear Diffusion of T7 DNA Polymerase: Thioredoxin is Required to Maintain Close Contact with DNA**
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The bacteriophage T7 DNA polymerase consists of a tight, 1:1 complex of T7 gp5, encoded by the phage, and thioredoxin, produced by the *E. coli* host. In the absence of thioredoxin, gp5 is capable of adding only a few nucleotides to the 3′ end of a primer before dissociating from the primer-template. But when complexed with thioredoxin, gp5 becomes highly processive, capable of polymerizing thousands of nucleotides complementary to the template strand. The mechanism by which thioredoxin acts as a processivity factor to gp5 is not fully understood. To understand the role of the thioredoxin in stabilizing polymerase-DNA interactions, we use a single-molecule imaging approach to observe individual, fluorescently labeled T7 DNA polymerase complexes diffusing along double-stranded DNA. Our results show that the average diffusion coefficient of T7 DNA polymerase complexes is insensitive to ionic strength and does not exceed the theoretical diffusion limit for a protein that tracks the helical pitch and rotates as it diffuses along the DNA helix. These results suggest that the T7 DNA polymerase slides along the DNA, remaining tightly bound to the DNA and tracking the helical pitch. However, the mean diffusion coefficients for fluorescently labeled T7 gp5 in the absence of thioredoxin increase with salt concentration, and exceed the theoretical limit for a protein tracking the DNA helix. Upon addition of unlabeled thioredoxin, the mean diffusion coefficient is restored to the value observed for the labeled T7 DNA polymerase, and becomes salt independent. These observations indicate that, in the absence of thioredoxin, T7 gp5 intermittently dissociates from the DNA as it diffuses, and that thioredoxin binding suppresses microscopic hopping on and off the DNA.

**2149-Pos Board B119**
**Protein mediated bridging motifs: A key mechanism in biopolymer organization**
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The protein-mediated bridging motif is ubiquitous and essential for shaping cellular structures in all organisms. In this paper, we dissect the bridging motif for a model system: the Heat-stable Nucleoid-Structuring protein (H-NS). We present data from two complementary single-molecule assays that probe the H-NS-DNA interaction: a dynamic optical-trap-driven unzipping assay and an equilibrium H-NS-mediated DNA looping assay. To quantitatively analyze and compare these assays, we develop a general theoretical framework for describing the bridging motif. The interplay between these experiments and our theoretical model not only infers the effective interaction free energy, the bridging conformation and the duplex-duplex spacing, but also reveals a second, unresolved, cis-binding motif that challenges our current understanding of the role of bridging proteins in chromatin structure. The theoretical framework we have developed is generally applicable to the description of protein-mediated bridging motifs in chromatin and cytoskeletal organization.

**2150-Pos Board B120**
**Tyrosyl-DNA Phosphodiesterase Binds Nucleic Acids Preferentially At The 3′ End**
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Tyrosyl-DNA Phosphodiesterase (Tdp1) is an enzyme that catalyzes the hydrolysis of 3′ phosphotyrosyl bonds. These linkages are formed in vivo following the DNA processing activity of topoisomerase I (Top1). In this study we have investigated the binding preference of Tdp1 for the 3′ or 5′ end of DNA. A 15 base deoxyoligonucleotide was labeled at either the 3′ or 5′ end with fluorescein attached via a phosphodiester or phosphothioate bond. Tdp1 was able to remove the fluorescein at the 3′ but not at the 5′ end when attached via a phosphodiester bond but not with a phosphothioate bond. Using fluorescence anisotropy we measured the binding of Tdp1 to these oligonucleotides. Tdp1 bound the 15mer with a 3′ fluorescein phosphothioate linkage 10 fold tighter than the 15mer with a 5′ fluorescein phosphothioate or phosphodiester linkage. No binding was observed to the 15mer with a 3′ fluorescein phosphodiester linkage due to the cleavage of the fluorescein. The higher binding affinity with a fluorescein compared to an OH at the 3′ end suggests Tdp1 has a preference for a large group in that position. The fluorescein at the 3′ or 5′ end was adjacent to a guanine residue that resulted in a quenching effect. Time resolved fluorescence studies showed the 3′ end was protected from quenching significantly more than the 5′ end when bound by Tdp1. Finally we immobilized a 14 base oligonucleotide with either a free 3′ or 5′ phosphate group on a Biacore sensor chip. Tdp1 bound to the oligonucleotide with the 3′ phosphate end free rapidly reaching a steady state and with a $K_d$ of 50 nM. No binding was observed when the 5′ end was free. These data are consistent with Tdp1 binding preferentially to the 3′ end.

**2151-Pos Board B121**
**A Label-Free, Force-Based Microarray Sensor**
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Within the past 15 years, force spectroscopy on single molecules has evolved from first proof-of-principles to a sophisticated method for the investigation of mechanics, folding kinetics, and complex formation of biomolecules. The quantification and characterization of molecular interactions is key to pharmaceutical and medical research, since it facilitates the development of

therapeutics and serves as the basis of many diagnostic techniques. Force-based ligand detection offers many advantages over conventional approaches based on the thermodynamics of the interaction. It is label-free and permits crowded ambients. However, it requires very expensive equipment as well as labor intensive and time-consuming protocols. More severely, since force spectroscopy is inherently limited by thermal fluctuations, a molecular complex under investigation has to be probed thousands of times in order to achieve sufficient force resolution. This confines its application to low throughput formats. Here we present a high-throughput force spectroscopy approach in a parallel format, which in addition allows the detection of subtle changes in mechanical stability below the ones e.g. caused by a single base-pair mismatch in dsDNA. A very low affinity ATP selective DNA aptamer was implemented into a microarray compatible differential force detector design, wherein the relative stability of an aptamer·ligand complex is probed against a constant dsDNA reference complex. We found that the label-free assay selectively quantifies the concentration of ATP and that it reliably operates in a challenging, molecular crowded environment. The simplicity of the assay qualifies it as a tool that can be used in any laboratory equipped with basic fluorescence microscopy.

**2152-Pos Board B122**

**Visualization of Force-Mediated Looping Dynamics of a Single DNA Molecule by the E. coli protein FIS**

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Various experiments have suggested that DNA is ''looped'' by proteins, such as the E coli nucleoid associated protein FIS, in a concentration- and force-dependent manner. However, there has been no direct evidence that discrete DNA condensation domains are formed by FIS, until now. Using a combined magnetic tweezers/fluorescence microscopy apparatus, we have measured the dynamics and visualized the formation of discrete condensation domains in a single DNA molecule by FIS. Visualization was achieved by binding a gfp-FIS conjugate to lambda-DNA at concentrations higher than 1uM. The force dependence and rate of condensation are demonstrated.

**2153-Pos Board B123**

**Distinguishing Dual DNA Binding Modes of Actinomycin D using Optical Tweezers**

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Actinomycin D (ActD), the first antibiotic which exhibited anti-tumor activity, was initially believed to bind double stranded DNA (dsDNA) through intercalation. Later it was shown to bind single stranded DNA (ssDNA) with an order of magnitude higher affinity. ssDNA binding can be extremely important in inhibiting replication of viruses that replicate through ssDNA templates such as HIV. While these two binding modes can be separately quantified by studying binding to specific substrates, it is very difficult to determine the mode of binding to polymeric DNA. DNA stretching studies can precisely quantify intercalation by measuring the increase in DNA length upon intercalation. However, ssDNA binding also increases DNA length. Therefore, we have developed a method that combines the measured increase in DNA length with the overall DNA melting free energy change, allowing us to simultaneously determine ssDNA binding and intercalation as DNA is stretched. Using this method, we were able to distinguish between dual binding modes of ActD. We determined that the ssDNA binding of ActD ($K_{ss} \sim 10^8\ M^{-1}$) is much higher than its binding to dsDNA ($K_{ds} \sim 10^6\ M^{-1}$) for long polymeric DNA. We also determined the ssDNA and dsDNA binding site size, which are 3 bases and 6 base pairs, respectively.

**2154-Pos Board B124**

**Inducer Effects on Lac Repressor-Mediated DNA Loops: Single-Molecule FRET Studies**

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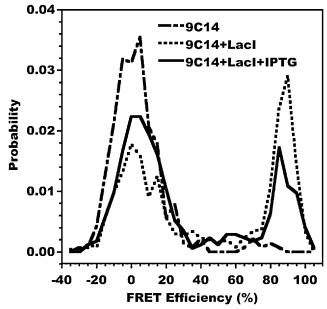
The *Escherichia coli* LacI protein represses the *lac* operon by blocking transcription. Tetrameric LacI binds simultaneously to a promoter-proximal DNA operator and an auxiliary operator, and the resulting DNA loop increases the efficiency of repression. A hyperstable closed-form LacI-DNA loop was previously shown to be formed on a DNA construct (9C14) that includes a sequence-directed bend flanked by operators. Previous bulk and single molecule fluorescence resonance energy transfer (SM-FRET) experiments on dual fluorophore-labeled 9C14-LacI loops demonstrate that LacI-9C14 adopts a single, stable, rigid DNA loop conformation, despite the presence of flexible linkers in LacI. Here, we characterize the LacI-9C14 loop by SM-FRET as a function of inducer isopropyl-β,D-thiogalactoside (IPTG) concentration. Energy transfer measurements reveal partial but incomplete destabilization of loop formation by IPTG, with no change in the energy transfer efficiency of the remaining looped population.




Models for the regulation of the *lac* operon often assume complete disruption of LacI-operator complexes upon inducer binding to LacI. Our work shows that even at saturating IPTG there is still a significant population of LacI-DNA complexes in a looped state, in accord with previous in vivo experiments that show incomplete induction.

**2155-Pos Board B125**

**DNA Structure Selectivity of *Escherichia coli* versus *Thermus aquaticus* DNA Polymerase I**

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Understanding substrate selection by DNA Polymerase I is important for characterizing the balance between DNA replication and repair for this enzyme *in vivo*. Due to their sequence and structural similarities, Klenow and Klentaq, the ''large fragments'' of the Pol I DNA polymerases from *Escherichia coli* and *Thermus aquaticus*, are considered functional homologues. We have examined the DNA binding thermodynamics of Klenow and Klentaq to different DNA structures: single-stranded DNA (ss-DNA), primer-template DNA (pt-DNA), and double-stranded DNA (ds-DNA). The DNA binding affinity trend for Klenow from weakest to tightest binding is ds-DNA $<$ pt-DNA $<$ ss-DNA. This is in contrast to Klentaq's DNA binding trend: ss-DNA $<$ pt-DNA $\approx$ ds-DNA. Both Klenow and Klentaq released more ions when binding to pt-DNA and ds-DNA than when binding to ss-DNA in KCl buffer. $\Delta Cp$ is the temperature dependence of the enthalpy of a reaction. Both of these non-sequence specific binding proteins exhibit relatively large heat capacity changes ($\Delta Cp$) upon DNA binding. $\Delta Cp$ values for binding of Klenow and Klentaq to the different DNA structures do not follow the same patterns as the $\Delta G$ values for binding, suggesting the balance of electrostatic versus hydrophobic interactions in the binding interfaces also differ between the two species of polymerase. It is also found that $Mg^{2+}$ significantly shifts the ds-DNA binding affinity of Klenow, but not Klentaq. $Mg^{2+}$ may be shifting the partitioning between the polymerization and editing sites on Klenow. The differences in DNA structural selectivity of the two polymerases suggest that the *in vivo* functions of these two supposedly homologous polymerases are different, and that Taq polymerase is more likely to be involved in ds-break repair and end-preservation *in vivo*. Funded by the NSF and the Louisiana Biomedical Research Network.

**2156-Pos Board B126**

**Modeling The Behavior Of DNA-Loop-Extruding Enzymes**

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Condensin proteins are large complexes belonging to a family of ATP hydrolyzing proteins known as SMC (Structural Maintenance of Chromosomes). Condensins are believed to play a vital role in chromosomal assembly and segregation in eukaryotic cells but the details of their function along chromatin are poorly understood. Here, we propose a model to describe the behavior of DNA-loop-inducing proteins, such as type I restriction enzymes, which we believe can be used to understand condensin's function. We assume an effective motor behavior for these enzymes in which the bias of the two dimer heads is to travel away from each other, which results in loop formation along the DNA lattice. Processivity causes the enzymes to stack on top of each other. We further discuss the results of theory and computer simulations for different values of motor bias and processivity.

**2157-Pos Board B127**

**Klenow and Klentaq-DNA Binding: the 'Glutamate Effect' is Primarily an Osmotic Effect**

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DNA binding by Klenow (*E.coli*) and Klentaq (*T.aquaticus*) DNA polymerases has been studied as a function of monovalent salt concentration, pH and osmotic stress. We previously showed that DNA binding resulted in the net release of 4.5~5 ions from Klenow and 3~3.5 ions from Klentaq. Here, we report