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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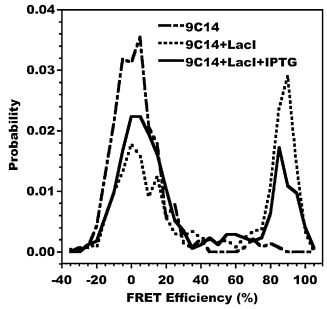
The *Escherichia coli* LacI protein represses the *lac* operon by blocking transcription. Tetrameric LacI binds simultaneously to a promoter-proximal DNA operator and an auxiliary operator, and the resulting DNA loop increases the efficiency of repression. A hyperstable closed-form LacI-DNA loop was 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. ΔCp is the temperature dependence of the enthalpy of a reaction. Both of these non-sequence specific binding proteins exhibit relatively large heat capacity changes (ΔCp) upon DNA binding. ΔCp values for binding of Klenow and Klentaq to the different DNA structures do not follow the same patterns as the ΔG 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