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.

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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.

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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.

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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.

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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