DNA stabilized by intercalation of conserved proline residues located on two β-ribbon arms that wrap around the DNA.
Previous stopped-flow and laser T-jump measurements on IHF binding to its cognate H'site revealed that DNA bending in the complex occurs on ~1-10 ms, similar to the time-scales for thermal disruption of a single base pair in B-DNA. Here we find that inserted mismatches that increase the DNA flexibility at the site of the kinks accelerate the bending rates by nearly the same factor as the corresponding increase in binding affinity. On the other hand, modifications in DNA away from the site of the kink, as well as mutations in IHF, designed to perturb specific protein-DNA contacts, leave the bending rates unchanged despite a ~60-100-fold decrease in the binding affinity. These results support our earlier conclusion that in the transition state ensemble separating the nonspecific from the specific complex the DNA is bent/kinked, but protein-DNA interactions that stabilize the complex have not yet been made.
Our measurements also reveal a rapid (~100 microseconds) phase in the bending kinetics. In contrast to the relaxation rates for the slow phase, which are affected by modifications in the DNA at the site of the kinks, the relaxation rates for the fast phase appear to be unaffected. This rapid phase may correspond to the wrapping/unwrapping of the β-arms of the protein in a nonspecific binding mode, as IHF scans potential binding sites on genomic DNA.

3442-Pos
Parallel Single-Molecule Study of DNA Repressor Kinetics
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In a bottom up approach to understanding molecular motors, a synthetic protein-based molecular motor, the ''tumbleweed'', is being designed and constructed [1]. This design uses three ligand dependent DNA repressor proteins to rectify diffusive motion of the construct along a DNA track. These proteins are MetJQ44K, TrpR, and DtxR. To predict the behavior of this artificial motor one needs to understand the binding and unbinding kinetics of the repressor proteins at a single-molecule level. An assay, similar to tethered particle motions assays [2], is used to measure the unbinding rates of these three DNA repressor proteins. In this assay the repressor is immobilized to a surface in a microchamber. Long DNA with the correct recognition sequence for one of the repressors is attached to a streptavidin-coated microsphere. As the DNA-microsphere construct diffuses through the microchamber it will sometimes bind to the repressor protein. Using brightfield microscopy and a CCD camera the diffusive motion of the microsphere can be characterized and bound and unbound states can be differentiated. On the order of ten microspheres can be easily visualized at one time allowing single-molecule measurements to be done in parallel. The resulting kinetic measurements are compared to bulk binding kinetics measured in a QCM device.
[1] EHC Bromley et al. 2009. The Tumbleweed: Towards a synthetic protein motor. HFSP Journal. 3:204-212.
[2] F Vanzi et al. 2006. Lac Repressor hinge flexibility and DNA looping: single molecule kinetics by tethered particle motion. Nuc. Acids. Res. 34:3409-3420.

3443-Pos
Determination of Protein Sliding and Hopping Kinetics Along DNA using Brownian Dynamics Simulations
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Recent single-molecule tracking experiments have observed diffusion of a protein, such as LacI, along DNA and reported its 1D diffusion coefficient (D_1) in the range of 10^2 nm^2/s to 10^5 nm^2/s in the timescale of seconds. These studies, however, lack the temporal resolution to capture the protein's sliding and hopping kinetics since the sliding or dissociation time has been estimated to be on the order of several milliseconds. A protein's dissociation time from nonspecific DNA and its sliding diffusion coefficient ($D_{sliding}$) are important parameters for understanding the mechanisms governing protein interaction with non-specific DNA and quantification of facilitated diffusion. Here we report on simulations, on the timescale of seconds, of proteins interacting with nonspecific DNA subject to alternating 1D (sliding) and 3D (hopping) diffusive processes with initial rates taken from literature. Comparison with the aforementioned experimental results (D_1 in the timescale of seconds) permits calculation of an upper bound for the dissociation time and $D_{sliding}$ by analyzing the mean square displacements of the simulated individual trajectories.

3444-Pos
Single Molecule Analysis of Yeast Rrp44 Exonuclease Reveals a Spring-Loaded Mechanism of RNA Unwinding
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The eukaryotic exosome catalyzes a series of reactions such as RNA processing and decay. Rrp44 is a key catalytic subunit of the yeast exosome complex and enables multi-enzymatic activities, including endoribonuclease, exoribonuclease and duplex unwinding. Its exoribonuclease and unwinding activities are indispensable for the complete degradation of mRNA that forms a variety of secondary structures. It is, however, unknown how the unwinding activity is coordinated with the exonuclease one. We used single-molecule fluorescence techniques to investigate the mechanism of unwinding by Rrp44 in real time. Surprisingly, we found that Rrp44 does not unwind the RNA duplex each time it digests a single nucleotide off the 3'end of the single stranded RNA tail. Instead, it accumulates elastic energy during multiple steps of RNA digestion and unwinds several basepairs simultaneously. The kinetic analysis of each unwinding step shows that RNA unwinding, not RNA digestion, determines the overall RNA degradation rate. A series of control experiments varying the RNA sequence, salt concentrations and temperature, and the use of hybrid RNA/DNA duplex demonstrate that the unwinding step size is determined by the physical properties of the enzyme itself, not by the duplex stability. Our studies represent the first example of a multiple hierarchy of stepping for an exonuclease.

3445-Pos
Design and Construction of a DNA Nanostructure for Direct and Dynamic Measurement of DNA Bending by DNA-Binding Proteins
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We report the design and construction of a nanostructure that makes it feasible to measure bend angles of short DNAs using conventional fluorescence microscopy. The nanostructure is comprised of a double-stranded DNA linker ~50 bp long, with a pair of non-complementary 5 nt sequences at each end, which bind to and bridge between two DNA nanotubes in a specific way. The DNA nanotubes are 10 double-helices in circumference and ~5um long. The resulting structure is thus a very stiff polymer with only one flexible point at the linker position. When electrostatically adsorbed onto a supported lipid bilayer, the nanostructures were effectively constrained to diffuse two dimensions, allowing direct visualization and measurement of the bend angle of the linker DNA. Our data show that this is a very promising approach for direct and dynamic measurements of DNA bending induced by DNA-binding proteins.

3446-Pos
Single Molecule FRET Observations of Pre-MRNA Splicing
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The spliceosome is the snRNA-protein machine that is responsible for the precise excision of introns from pre-mRNAs in eukaryotes. The steps required for the catalytic activation of the spliceosome require a dynamic set of ATP dependent RNA-RNA and RNA-protein interactions. To begin to dissect the kinetic and conformational requirements for pre-mRNA positioning during spliceosome assembly, we have developed a single molecule FRET (smFRET) splicing assay in which donor and acceptor fluorophores are placed at various positions within the pre-mRNA. By using continuous excitation of the donor fluorophore to obtain short high resolution FRET trajectories of single pre-mRNAs in splicing extract we have been able to observe time- and ATP- dependent conformational dynamics during spliceosome assembly. In a complimentary approach we have used pulsed excitation of the donor fluorophore to limit the effects of photobleaching on our observation window. Using this complimentary approach we are now able to visualize a single pre-mRNA in the time scales required for an in-vitro splicing assay. We have seen that the pre-mRNA is taken through a series of largely reversible conformational steps throughout every step of the splicing reactions. Labeling spliceosomal proteins and RNAs in extract will give us dynamic information regarding their assembly and regulation.