The rotor bead technique introduced here allows tracking of the complete three-dimensional trajectory of a dsDNA translocase in action. It also permits the application of torque in a laser tweezers apparatus using commercially-available microspheres.

1147-Plat
Reeling in DNA One Base at A Time: pcrA Translocation Coupled to DNA Looping Dismantles RecA Filaments
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The mechanism of helicase translocation on DNA remains controversial and the translocase activity driving their non-canonical functions such as protein displacement is poorly understood. Here, we used single molecule fluorescence assays to study a prototypical superfamily 1 helicase, *Bacillus stearothermophilus* PcrA, and discovered a progressive looping of ssDNA that is tightly coupled to PcrA translocation on DNA. Variance analysis of hundreds of looping events by a single protein demonstrated that PcrA translocates on ssDNA in uniform steps of 1 nt, reconciling discrepancies in previous structural and biochemical studies. On the forked DNA, rather than acting on the leading strand to unwind the duplex, PcrA anchored itself to the duplex junction and reeled in the lagging strand using its 3'-5' translocation activity. PcrA maintained the open conformation, not the closed conformation observed in crystallographic analysis, during looping-coupled translcation. This activity could rapidly dismantle a preformed RecA filament even at 1nM PcrA, suggesting that the translocation activity and structure-specific DNA binding are responsible for removal of potentially deleterious recombination intermediates.

1148-Plat
Protein-Mediated DNA Loops are Resistant to Competitive Binding
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The lac Repressor protein (LacI) is a canonical genetic regulatory protein. It represses transcription of the lac operon in E. Coli by simultaneously binding to two distant operator sites on the bacterial DNA and bending the intervening DNA into a loop. A set of substrate DNA constructs with intrinsic A-tract bends have been engineered by Mehta and Kahn, which were optimized to form hyperstable loops. We present single-molecule measurements of LacI-mediated loop formation and breakdown rates on these optimized DNA constructs and demonstrate that repeated formation and breakdown of the loops does not cease in the presence of 100 nM of free competitor DNA. While this observation dovetails with bulk competition assays in which the presence of competitor DNA disrupts the looped complexes only very slowly, our measured loop lifetimes of minutes disagree with an inferred lifetime of days from the bulk assays. We conclude that the LacI-DNA complex can exist in some non-looped conformation, which can re-loop, but is unexpectedly resistant to competition. We discuss possible scenarios for such a conformation in light of the data.

1149-Plat
Single Molecule Analysis of Substeps in the Mechanochemical Cycle of DNA Gyrase
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DNA gyrase is a molecular motor that harnesses the free energy of ATP hydrolysis to introduce negative supercoils into DNA. We have characterized the structural dynamics of processive supercoiling using a real-time single molecule assay in which DNA gyrase activity drives the directional, stepwise rotation of a submicron rotor bead attached to the side of a stretched DNA molecule. We are able to directly observe rotational pauses corresponding to rate-limiting kinetic steps under varying [ATP], and have used simultaneous measurements of DNA twist and extension in order to characterize transient supercoil trapping and DNA compaction during the reaction cycle. We have mapped out structural intermediates of the DNA:gyrase complex on a twist-extension plane, and have characterized transitions between these states driven by chemical events such as the cooperative binding of ATP. These measurements motivate several revisions to previous models based on lower resolution assays [1], and we will present our results in the context of a new branched kinetic model for the mechanochemical cycle. We are now using theoretical calculations together with measurements of force-dependent changes in extension in order to test specific geometric models for structural intermediates, and we have begun to analyze structure-function relationships using single-molecule analysis of gyrase fragments.
[1] Jeff Gore, Zev Bryant, Michael D. Stone, Marcelo Nollmann, Nicholas R. Cozzarelli, Carlos Bustamante, "Mechanochemical analysis of DNA gyrase using rotor bead tracking", Nature, 439 (2006)

1150-Plat
Two Structurally Different Families of DNA Base Excision Repair (BER) Proteins Diffuse Along DNA to Find Intrahelical Lesions
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Base excision repair (BER) proteins, endonuclease III (Nth) and VIII (Nei) from E. *coli* represent two distinct glycosylase families, which recognize and remove damaged DNA bases. One mechanism by which these glycosylases scan for DNA lesions is through a simple, one-dimensional diffusive search. To characterize this search mechanism, we have developed a single molecule assay in near TIRF to image Qdot-labeled, His-tagged Nth and Nei proteins interacting with YOYO-1 stained λ-DNA molecules elongated by hydrodynamic flow between 5μm silica beads. With an *in vitro* glycosylase activity assay, we confirmed that neither YOYO-1 stained DNA nor Qdot labeling significantly affects glycosylase activity. By imaging individual DNA "tightropes", we observed Qdot-labeled glycosylases interacting with DNA by either binding to or diffusing on DNA. With increasing ionic strength (50-500mM Kglutamate), although fewer glycosylases interacted per unit length of DNA, a greater fraction diffused along the DNA. At physiological ionic strength, (150mM KGlu) both Nth and Nei scan DNA for as much as 10 sec with a diffusion constant of ~1.5 x 10^5 bp^2 sec^{-1}, approaching the theoretical limit of rotational diffusion about the DNA helix. At these rates, the activation barrier for rotational diffusion of 0.7 K_bT is slightly below the maximum of ~2 K_bT for efficient target location. We observe no significant difference between Nth and Nei in the rate or mode of their DNA lesion search mechanism. Interestingly, at elevated ionic strengths, both families of glycosylases scan above the theoretical limit for free rotational diffusion (>5 x 10^5 bp^2 sec^{-1}). Therefore, the DNA/glycosylase interface may be optimized for physiological ionic strength, above which the glycosylase search mechanism shifts from rotational diffusion to a one-dimensional diffusion without rotation.

1151-Plat
Target-Site Search of DNA-Binding Proteins
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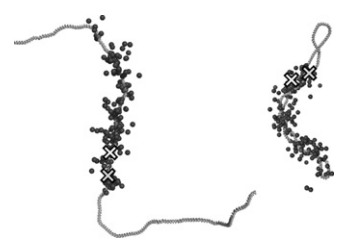
Gene regulatory proteins find their target sites on DNA remarkably fast; the experimental binding constant for *lac* repressor is three orders of magnitude higher than predicted by free diffusion alone. It has been proposed that nonspecific binding aids the search by allowing proteins to slide and hop along DNA. We develop a reaction-diffusion theory of protein translocation that accounts for transport both on and off the strand and incorporates the physical conformation of DNA. For linear DNA modeled as a wormlike chain, the distribution of hops available to a protein exhibits long, power-law tails. As a result, the long-time displacement along the strand is superdiffusive. Our analysis predicts effective superdiffusion coefficients for given nonspecific binding and unbinding rate parameters. Translocation rates experience a maximum with salt concentration (i.e., binding rate constant), which has been verified experimentally. Simulated protein trajectories on DNA (see figure) agree with our theoretical predictions of superdiffusive transport. Our analytical theory allows us to predict the binding and unbinding rate parameters that optimize the protein translocation rate and the efficiency of the search. Finally, we use our theory to predict rates of target site localization under various experimental conditions.



1152-Plat
Illuminating the DNA Binding Behavior of Mitochondrial Transcription Factor A
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