

long steps are not prevented by SSBs. Step size distributions for HJs containing SSB were obtained and such characteristics of these distributions as the mean hop size and the distributions width remain virtually the same as in the designs without a break. Lifetimes of folded states were found to be very similar to that in control, just slightly increasing around the SSB in the AT-rich design. We also studied an immobile HJ with a SSB at the junction. Its arms remain in antiparallel orientation, very similarly to our previous studies. Supported by the NSF grant PHY-0615590 (YLL).

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Nucleocapsid Protein Locally Melts The IIB Region Of Hiv-1 Rre Rna, But Rev Protein Does Not: A Single-molecule Spectroscopic Study Yu-shan Yeh¹, Hui Wang¹, Paul F. Barbara¹, Matthew D. Daugherty², Alan D. Frankel², Karin Musier-Forsyth³.

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The focus of this work is to develop a single molecular-level understanding of how the binding of two important viral proteins of human immunodeficiency virus type-1 (HIV-1), Rev and nucleocapsid (NC) proteins, locally change the secondary structures of the IIB stem-loop region of the Rev-Responsive Element (RRE) of the HIV-1 viral genomic RNA. Rev and NC represent two types of nucleic acid-binding proteins in HIV with distinct structures, behaviors and functionalities. Rev is a sequence-specific RNA-binding protein that binds to stem IIB and other regions of the RRE, facilitating nuclear export of unspliced HIV RNAs. In contrast to Rev, NC is a multifunctional protein that plays a role in almost every step of the retroviral life-cycle. NC can bind both DNA and RNA hairpin structures using its CCHC-type zinc fingers and basic domains. Here we systematically investigate how the binding of Rev and NC on the 42-nt IIB RNA hairpin locally changes the RNA secondary structures using a single-molecule fluorescence resonance energy transfer (SM-FRET) approach. A series of RNA and DNA oligonucleotides containing appropriate dyes have been designed to probe the local melting of the IIB RNA hairpin using a single-molecule oligonucleotide annealing assay. Our study shows that NC locally melts the IIB RNA hairpin but Rev does not, illustrating how two proteins that use dramatically different nucleic acid recognition motifs can give rise to very different secondary structural changes upon RNA binding. This SM-FRET-based approach provides a unique way to gain insight into the secondary structural change of HIV viral RNAs induced by protein binding with minimal interference from protein aggregation.

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Single-pair Fluorescence Resonance Energy Transfer study of mononucleosomes dynamics

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Nucleosomes, the primary repeating unit of chromatin, package DNA by wrapping 147 base pairs tightly around an octamer of histone proteins in approximately 1.7 helical turns. In order to allow central nuclear processes to happen, such as DNA replication, recombination, repair and transcription, regulated exposure of the template DNA is required. Therefore, the nucleosome has to undergo certain conformational changes. One possible strategy for such a regulated exposure is the translocation or 'sliding' of nucleosomes along the DNA by the class of ATP-hydrolyzing enzymatic machines called chromatin remodelers.

We apply single pair-FRET for direct observation of intrinsic nucleosome dynamics as well as conformational changes of mononucleosomes induced by chromatin-remodeling complexes. The movement of DNA around the nucleosome surface is tracked in the presence and absence of remodeler by using mononucleosomes, which are reconstituted with a 200 bp long DNA containing the nucleosome positioning sequence 601 and a donor- and acceptor-dye pair at well-defined positions. Mononucleosomes are immobilized on DOPC-bilayer functionalized quartz slides and visualized by Total Internal Reflection Microscopy.

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Unraveling Nucleosome Dynamics Using Fluorescence Fluctuation Analysis

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Nucleosomes are the smallest repeating units of eukaryotic chromatin. Target DNA sites for various biological processes are often buried inside these nucleosomes. There are various mechanisms by which DNA accessibility is in-

creased. Recent studies show that DNA spontaneously and transiently unwraps from the histone core, presenting itself to the protein machineries. We have studied this dynamics using fluorescence correlation analysis. Our approach is to use a FRET construct where the acceptor (Cy5) is on the histone while the donor (Cy3) is moved along the length of the DNA, starting from the 5' terminus all the way to the center of the dyad axis. We have used a combination of experimental FCS techniques and Monte Carlo simulations to determine the timescales of the nucleosome wrapping-unwrapping process. Conventional FCS methods face the challenge of separating the kinetic contributions from the diffusion contributions to the autocorrelation functions. Here, we will present and discuss a variety of approaches aimed to overcome these difficulties, including the analysis of the donor-acceptor cross-correlation and correlation of the generalized polarization function.

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A Polarized View Of DNA Under Tension

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Polarized fluorescence measurements are valuable tools for determination of local structure and dynamics of biomolecules. In many applications, a great difficulty is to orient the biomolecules in a fixed and well-defined orientation, such that absolute measures of the directions of the absorption and emission dipole moments are attainable. Often alignment in flow or in a compressed gel is used, but this yields heterogeneity in the orientations. Here we present a novel and different approach: we use two optical tweezers to extend and align a piece of DNA and measure, at the same time, the polarization of the fluorescence of intercalating dye molecules (YOYO). This approach not only provides superior control of the orientation of the DNA but also of tension and consequently its conformation. By using this approach, we resolve an inconsistency in reports on the tilt angle of intercalated dyes with respect to the DNA long axis. We find that intercalated dyes are on average oriented perpendicular to the DNA, yet undergo fast dynamics on the fluorescence time scale. In addition, we assess the structural changes occurring in and beyond the overstretching transition of double-stranded DNA, during which the base pairs gradually melt. We observe that at low forces, when the DNA is shorter than its contour length, the alignment of the dyes increases with force, which can be well-described with a simple Monte-Carlo model of DNA flexibility. During the overstretching transition, the orientation of the dipoles does not change, which we attribute to the intercalating dyes acting as local stabilizers of the helical DNA structure. Beyond the overstretching transition, the dipole moments rotate towards the DNA's long axis, which we attribute to shearing of the locally stabilized double-stranded structure, leading to tilted intercalators and bases.

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A Novel Approach to the Detection of DNA-Enzyme Interaction Processes at a Single-Molecule Level

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A fundamental problem in both molecular biology and biophysics is how DNA-binding proteins find specific sites among huge amounts of non-specific (chromosomal) DNA. This in turn requires experimental procedures to elucidate the mechanisms of both non-specific and sequence-specific interactions of proteins with DNA. Current techniques are incapable of directly observing these interaction processes. We recently constructed a setup (See Image) for tracking trajectory of single reactant DNA molecules during enzymatic reaction. It uses a microfluidic flow system to pick reacting DNA molecules (previously tagged at one end by a quantum dot) out of the crowd of molecules. Restriction enzymes Not I, Apa I and EcoR I with their target and non-target DNA were analyzed. Quantitative assessments of their interaction time were achieved by

