recombination and repair. In the present work we are investigating mechanical properties of torsionally-denatured DNA at the single-molecule level using an angular optical trap. While applying a constant tension to a DNA molecule, we simultaneously measure the extension change and torque as the DNA is wound up and denatured. We will present measurements on both tensile and torsional properties of denatured DNA. We will also discuss the implications of our findings with respect to previous theoretical work.

2420-Pos

The Rule of Seven Revealed by Observing DNA Annealing in a Nanocontainer

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Although the melting temperature (T_m) of DNA can be predicted with great accuracy, little is understood about the basic rates governing the helix-coil transitions between two strands of DNA. Here we adapt a porous vesicle encapsulation method with single-molecule fluorescence to measure these rates directly for a 9 bp DNA duplex (T_m=23°C) and characterize their variation with mismatched basepairs. A single basepair mismatch can cause up to three orders of magnitude variation in duplex stability. Surprisingly, we found that the rate of DNA annealing shows an abrupt 100 fold change depending on whether there are 7 or more contiguous bp or not (~10^6 M^{-1} sec^{-1} vs. ~10^4 M^{-1} sec^{-1}). Similar results were obtained for a microRNA seed with 7 bp match to *p53* and 6 bp match to *LIN28* gene sequences. Our results suggest a phenomenological cooperativity of 7 basepairs during Watson-Crick sequence recognition, with fundamental implications in nucleic acid pairing processes such as microRNA targeting and silencing in posttranscriptional regulation, and have practical implications for DNA microarray applications.

2421-Pos

Probing DNA Sequence Heterogeneity thorough Single-Molecule Studies of Supercoiling

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DNA has sequence-dependent mechanical properties that play a critical role in many biological processes, including initiation of DNA replication, gene expression, and interactions of DNA-binding proteins with their targets. Recent single-molecule experiments, in which a single molecule of DNA is stretched and/or twisted, have quantified aspects of DNA's mechanical properties, such as its bend and twist moduli. However, these experiments generally treat DNA as a homogeneous molecule; thus, they are insensitive to the effects of DNA sequence heterogeneity. To sense sequence-dependent effects, we have built a novel instrument that combines fluorescent imaging with magnetic methods for manipulating DNA. With this instrument we have investigated the locations of plectonemic branches on a long supercoiled molecule: since plectonemes are highly bent structures, we hypothesize that they will preferentially appear at easily-bendable or intrinsically-bent locations. We present data on plectoneme localization within a twisted lambda DNA molecule, and interpret the data within the context of theoretical predictions of DNA's sequence-dependent mechanical properties.

2422-Pos

Supercoiling Double-Stranded RNA

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Through a novel "polymerase-stall" labeling procedure, we have successfully generated torsionally constrained molecules of double-stranded RNA (dsRNA). We have anchored these molecules within a magnetic tweezers apparatus, and by rotating the magnets, induced both positive and negative supercoils within these molecules. Up to this point, only experimental data from supercoiling dsDNA has been available for testing current models of the elastic behavior of twist-storing polymers. Since dsRNA is an A-helix (whereas dsDNA is a B-helix), it has differing values for both bending and twisting stiffness, and thus provides a valuable second-case for the testing and refinement of these models.

Furthermore, dsRNA has important roles within biology, in its own right; not least among these, is that dsRNA is the central player in the gene-silencing pathway mediated through small interfering RNAs (siRNAs). The novel dsRNA substrates we have created, now pave the way for a more detailed understanding of the mechanistic action of the processes that constitute this pathway, at the single-molecule level.

2423-Pos

Mechanical Stabilisation of an Essential Subdomain of the Ribosome

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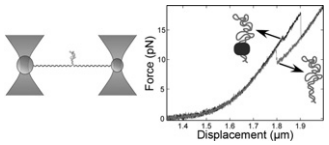
Whereas considerable information is available on ribosome structure and function, far less is known on how ribosomes are assembled. Our work focuses on a region of the large subunit that binds a number of proteins including L20, an early assembly protein that is essential for the binding of several other r-proteins. On the secondary structure of 23S rRNA this region appears as a long irregular stem, with L20 bound to the bottom. Like for many other ribosomal proteins, the effect of this binding on the structure of the target RNA is not known. By unwinding this region, using a single molecule trapping assay, we localize the L20 binding site within less than two base pairs and we show that L20 increases the stability of the bottom of the stem. Thus L20 acts as a clamp stabilizing the subdomain for later assembly steps. Our approach, which is the first study of this kind on RNA-protein interaction, should be applicable to other RNA-protein complexes.



2424-Pos

Simulated and Mechanical Unfolding of the Beet Western Yellow Virus −1 Frameshift Signal

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Mechanical unfolding of −1 frameshift signals such as RNA pseudoknots have aimed to test the hypothesis that the stability of the pseudoknot (PK) is directly correlated to the frameshifting efficiency. Here we report unfolding of the Beet Western Yellow Virus (BWYV) PK by optical tweezers complemented by computer simulations using steered molecular dynamics (SMD). Three BWYV PK scenarios were studied: the wild-type PK in the presence and absence of Mg^{2+}, and mutations of nucleic base C8 known to completely abolish −1 frameshifting by disrupting pseudoknot stability at the core of its structure. Despite significant differences in loading rates, we found the experimental and computational results to be remarkably consistent.

The SMD simulations provide a detailed sequence of molecular unfolding events that can be assigned to the force-extension profiles obtained with the optical tweezers. In the absence of Mg^{2+}, stretching of the PK using the optical tweezers does not result in the observation of any unfolding transitions, which is consistent with the SMD simulation that demonstrates the essential role of Mg^{2+} for the formation of a very strong salt bridge between G4, C5, G16, and C17 nucleotides. The C8 mutants, like wild-type unfolding in the absence of Mg^{2+}, unfold readily and at low force, consistent with the absence of any −1 frameshifting activity for these mutants.

2425-Pos

Ethanol Induced Shortening of dsDNA in Nanochannels

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The entropic confinement and manipulation of DNA in fabricated nanostructures has facilitated both the study of DNA-protein interactions and the polymer physics of DNA conformations in different solvent conditions and geometries. Moreover, it holds great promise as a powerful tool for rapid genomic sequencing. Ethanol precipitation is a common tool in molecular biology used to purify and concentrate DNA, typically in 70% (or greater) ethanol solutions. Even at lower ethanol concentrations, however, DNA has been shown to undergo a transformation from its physiological B-form to A-form, a shorter yet slightly less twisted molecular conformation. To examine this transition, we isolated individual YOYO-1 labeled λ-DNA molecules in 100nm×100nm nanochannels in 0, 20, 40 and 60% ethanol solutions. We observed a dramatic shortening in the mean measured lengths with increasing ethanol and a broadening of the distribution of measured lengths at the intermediate ethanol concentrations. These observed lengths are less than that of fully A-form λ-DNA, suggesting that other mechanisms are involved in shortening the observed molecules. First, the possible effect of ethanol dislodging of the intercolated fluorophores and subsequent shortening the observed molecule is discussed. Second, the