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

Ibrahim Cisse[1,2], Taekjip Ha[3,2].
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Center for the Physics of the Living Cell, Urbana, IL, USA, [3]Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

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

Jun Lin, Omar A. Saleh.
BMSE and materials Dept, university of california, Santa barbara, CA, USA.

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

Gary M. Skinner, Serge P. Donkers, Jan Lipfert, Nynke H. Dekker.
Delft University of Technology, Delft, Netherlands.

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.

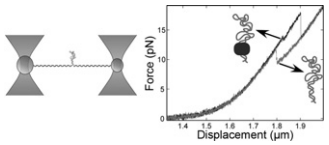
Furthermore, dsRNA has important roles within biology, in its own right; not least among these, is that dsRNA is the central player in the gene-silencing pathway mediated through small interfering RNAs (siRNAs). The novel dsRNA substrates we have created, now pave the way for a more detailed understanding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

Pierre Mangeol[1], Thierry Bizebard[2], Mathias Springer[2], Marc Dreyfus[2], Ulrich Bockelmann[1].
[1]ESPCI, Paris, France, [2]IBPC, Paris, France.

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

Katherine H. White[1], Marek Orzechowski[2], Dominique Fourmy[3], Koen Visscher[1].
[1]University of Arizona, Tucson, AZ, USA, [2]University of Basal, Basal, Switzerland, [3]ICSN-CNRS, Gif-sur-Yvette, France.

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

Gregory J. Gemmen[1], Walter W. Reisner[2], Jonas Tegenfeldt[3,4], Heiner Linke[3].
[1]Univ of Oregon, Eugene, OR, USA, [2]McGill University, Montreal, QC, Canada, [3]Lund University, Lund, Sweden, [4]University of Gothenburg, Gothenburg, Sweden.

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

substantial variations in intensity in our observed molecules at the higher ethanol concentrations are (i) suggestive of the higher order DNA conformations such as loops and toroids that have been observed in DNA dried on mica surfaces and (ii) in accord with the observation that the effective persistence length of DNA is reduced in ethanol solutions.

2426-Pos

Local Conformation of Confined DNA Studied using Emission Polarization Anisotropy

Fredrik Persson[1], Fredrik Westerlund[1], Anders Kristensen[2], Jonas O. Tegenfeldt[1].
[1]Gothenburg University, Gothenburg, Sweden, [2]Technical University of Denmark, Kongens Lyngby, Denmark.

When confined in nanochannels with dimensions smaller than the DNA radius of gyration, DNA will extend along the channel. We investigate long DNA confined in nanochannels, using fluorescence microscopy and intercalated dyes. Studies of the dynamics and statics of DNA in such nanoscale confinements as a function of e.g. degree of confinement and ionic strength have yielded new insights into the physical properties of DNA with relevance for applications in genomics as well as fundamental understanding of DNA packaging in vivo. Our work extends the field by not only studying the location of the emitting dyes along a confined DNA molecule but also monitoring the polarization of the emitted light. By measuring the emission polarized parallel and perpendicular to the extension axis of the stretched DNA, information on the local spatial distribution of the DNA backbone can be obtained. Comparing polarizations in two directions for DNA confined in channels of effective diameters of 85 nm and 170 nm reveals a striking difference. Whereas the DNA in the larger channels shows an isotropic polarization of the emitted light, the light is to a large extent polarized perpendicular to the elongation of the DNA in the smaller channels. We expect this technique to have a large impact on the studies of changes in DNA conformation induced by protein binding or during DNA compactation as well as in fundamental polymer physics studies of DNA in confined environments, for example in bacterial spores and viruses.

2427-Pos

Structure and Thermodynamics of ssDNA and dsDNA Near a Surface: a Coarse Grained Approach

Joaquin Ambia Garrido, Montgomery Pettitt.
University of Houston, Houston, TX, USA.

In the last two decades, new technologies have allowed us to measure properties of single DNA molecules in very accurate ways. At the same time, a number of theoretical models have been developed to understand the behavior of single stranded and double stranded DNA. These models have been shown to be accurate and relatively simple for very short systems of 6-8 base pairs. Comparatively less is known about the influence of a surface on the secondary structures of longer molecules important to many technologies. To gain insight into this situation we modeled DNA as a discretized worm-like chain; each link is considered a sphere of 6 base pairs in length for dsDNA and 1.5 bases for ssDNA. The chain is tethered to a surface by a fixed length, non-interactive 1 nm linker. Configurational sampling was achieved via Monte-Carlo sampling. Results on the average tilt are in agreement with all atom simulations. New insights into polyelectrolytes near surfaces are shown.

2428-Pos

Mechanical Constraints on Confined DNA

Benoît Knecht.
University of Heidelberg, Heidelberg, Germany.

A confined DNA molecule adopts various conformations, driven by entropy and constrained, in particular, by excluded volume interaction. But DNA also has some specific mechanical characteristics, such as twisting and bending elasticity. These properties influence the way DNA compacts itself inside the confined space, by favoring some conformations and impeding others. This situation is notably observed in E. Coli cells. Our model allows to simulate long polymer chains with given twist and bend elasticity constants, using the Monte Carlo method. Generating conformations with various twisting and bending rigidity gives detailed information on how DNA could be organized in such cells. By tuning the model with data from experiments on DNA mechanical properties, it becomes possible to make predictions on the DNA structure inside the cell nucleus.

2429-Pos

Self Organization in DNA-Loop-Extruding Enzymes

Elnaz Alipour-Assiabi, John F. Marko.
Northwestern University, Evanston, IL, USA.

We consider DNA-organizing molecular machines consisting of two coupled and oppositely directed motors which act to extrude loops from the double helix that they move along, while excluding one another sterically. In the case where these machines do not dissociate from the DNA (infinite processivity), the steady-state loop distribution is exponential and is described by an effective statistical-mechanical ensemble. However, if enzyme dissociation-rebinding occurs at any finite rate (finite processivity), the steady state qualitatively changes to a highly ordered "stacked" configuration with suppressed fluctuations, with tight hairpin-like condensation of the underlying DNA. This steady-state behavior can be understood via an approximate mapping to the restricted solid-on-solid model in an external field. Possible experimental realizations of these types of molecular machines are discussed, with a focus on type I restriction enzymes and condensin complexes.

2430-Pos

A Generalized Theory of DNA Cyclization and Loop Formation

David P. Wilson[1], Todd Lillian[1], Alexei Tkachenko[2], Noel C. Perkins[1], Jens-Christian Meiners[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Brookhaven National Laboratory, Upton, NY, USA.

We have developed a semi-analytic method for calculating the Stockmayer Jacobson J-factor for protein mediated DNA loops, as well as DNA ring cyclization. The formation of DNA loops on the order of a few persistence lengths is a key component in many gene regulatory functions. The binding of LacI protein within the Lac Operon of E.coli serves as the canonical example in which loop regulated transcription is understood. This fundamental looping motif consists of one protein simultaneously bound to two DNA operator binding sites. We explore as inputs the effect of sequence-dependent curvature and elasticity on the formation of DNA loops by constructing a Hamiltonian describing thermal fluctuations about the open and looped states. These fluctuations allow us to compute the entropic cost of loop formation, and thus allow a full computation of the free energy. Our work demonstrates that even for short sequences of the order one persistence length, entropic contributions are required to correctly compute the J factor.

We determine the lowest energy shape of the inter-operator DNA loop using a non-linear mechanical rod model under prescribed binding topologies (e.g, parallel and anti-parallel binding). Expanding about this shape allows us to calculate the J factors associated with parallel and anti-parallel binding topologies within the Lac system, and thus how entropy influences the most energetically favorable topology. The J factor can be used to compare the relative loop lifetimes of various DNA sequences, making it a useful tool in sequence design. Our work also allows the computation of an effective torsional persistence length, which demonstrates how torsion bending coupling affects the conversion of writhe to twist.

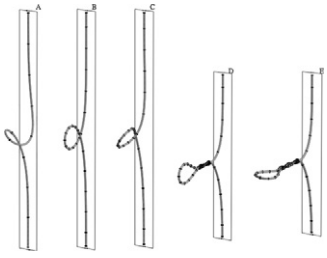
2431-Pos

Theory for the DNA Supercoiling Transition in Extension-Rotation Experiments

John F. Marko[1], **Sebastien Neukirch**[2].
[1]Northwestern University, Evanston, IL, USA, [2]UPMC Univ Paris 6, Paris, France.

Extension jumps were recently observed in single-molecule experiments where a DNA molecule (few kbp long) is held under tension while its ends are slowly rotated. For low rotation the molecule is believed to adopt (disordered) straight configurations and when a rotation threshold is reached the molecule jumps into a supercoiled phase: plectonemes arise. The transition is not continuous: the end-to-end extension of the molecule experiences an abrupt decrease.

We develop a theory where we compare the free-energies of the straight and supercoiled states. Care is taken with the energy of the supercoiled state where bending and twist energies for the plectonemes tip and the region joining the plectonemes and the ends of the molecules are included.



We find that the free energies of the straight and supercoiled states cross for a value n* of the imposed rotation. The extension jump is then given by the difference between the extension of the two states. Theoretically computed values compare well with experimental data.