frog Xenopus laevis during apoptosis. The two-electrode voltage-clamp technique was used to record endogenous ion currents in stage V or IV oocytes treated with staurosporine. We found that a sodium current was activated at voltages more positive than 0 mV with a mid point of the open-probability curve around +50 mV. Opening and closing kinetics were roughly exponential with time constants between 10 and 50 ms. The current was resistant to both 1 uM tetrodotoxin and 10 uM amiloride, while 200 uM verapamil in the bath solution completely blocked the current. Oocytes treated with both staurosporine and verapamil failed to upregulate the sodium current (measured in the absence of verapamil). We conclude that a verapamil-sensitive Na current is important in the apoptotic process in Xenopus oocytes.

2414-Pos

Photobiomodulation of Cellular Signalling and Apoptosis Induction in Human T Cells

Rahul P. Sinha, Gyongyver Katona, Magdalena Mocanu, Eugen Radu, Eva Katona.
Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Aiming to contribute to the understanding of molecular and cellular mechanisms involved in photobiomodulation, the present studies were undertaken to monitor short and long term laser irradiation effects in metabolically intact and metabolically impaired human T cells. We used AlGaInP/GaAs lasers with emission wavelengths in the range 600 - 900 nm and exposed T leukemia lymphoblasts and peripheral blood derived adherent and non-adherent mononuclear cells, cultured in normal and in energy/nutrient restriction caused stress conditions, to doses and irradiation regimes of therapeutic significance (total incident doses up to 15 μJ/cell). Energy/nutrient restriction was realized by serum starvation, glucose deprivation or blockade of glycolysis/oxidative phosphorylation. Selecting appropriate molecular reporters, we traced changes occurring in characteristics of cell signaling key players, and rates of cellular proliferation and apoptosis induction. Cell cycle progression, percentage of apoptotic/necrotic cells, and intracellular calcium and ERK phosphorylation levels, were assessed in single cell and cell suspension measurements. The data obtained by conventional, phase contrast, and fluorescence microscopy, steady-state fluorimetry, electrophoresis/imunoblotting, and flow cytometry demonstrate significant cell type, cell state, irradiation regime, radiation dose, radiation wavelength, and treatment duration dependent soft laser effects in human T lymphocytes and leukemia lymphoblasts.
Partial financial support of the Romanian Ministry of Education, Research and Innovation (grant 42139/2008 "REUMALAS") is gratefully acknowledged.

DNA, RNA Structure & Conformation II

2415-Pos

Real-Time Detection of Cruciform Extrusion by Single-Molecule DNA Nanomanipulation

Ramreddy Tippana.
Institute of Jacques Monod, Paris, France.

Cruciform extrusion in dsDNA can occur when a DNA palindrome is subjected to physiological levels of negative supercoiling. Here we use single-DNA nanomanipulation to explore the kinetic and structural properties of cruciform extrusion induced by negative supercoiling. Cruciform extrusion appears as an abrupt increase in the extension of negatively supercoiled DNA, and the amplitude of the change in extension is proportional to the number of bases in the cruciform. The kinetics of this two-state system, B-DNA and cruciform DNA, can be tuned by negative supercoiling which destabilizes the former and stabilizes the latter. The rate of extrusion is controlled by the size of the apical loop, decreasing as the loop size is increased from 5 to 8 bases. Cruciform rewinding is controlled by features in the stem. Perfect cruciforms will tend to extrude irreversibly, whereas shortening and addition of imperfections to a stem can render extrusion reversible. From measurements of the effect of torque on extrusion/rewinding kinetics we propose that in the transition state to cruciform extrusion the palindrome is unwound in the unpaired loop region of the cruciform. These results provide insight into the mechanism of cruciform extrusion and help to understand the potential role of these structures in processes of genomic instability as well as those underpinning the synthesis of non-coding RNAs.

2416-Pos

A Direct Observation of Highly Bent and Twisted DNA at the Single Molecule Level

Troy Lionberger[1], Davide Demurtas[2], Todd Lillian[1], Julien Dorier[3], Noel Perkins[1], Andrzej Stasiak[3], Edgar Meyhofer[1].
[1]Univ. of Michigan, Ann Arbor, MI, USA, [2]Ecole Polytechnique Fédérale de Lausane, Lausanne, Switzerland, [3]Univ. of Lausanne, Lausanne, Switzerland.

Many DNA-binding proteins interact with twisted or bent DNA. To characterize the activity of these proteins as a function of the torsional and bending stresses, we must first understand how these mechanical stresses affect the DNA tertiary structure (topology). To experimentally define this relationship on scales that are biologically relevant to DNA-binding proteins requires DNA molecules which stably maintain high degrees of stress and deformation on a length scale appreciably below the persistence length. DNA minicircles of ~100bp in size offer a unique opportunity to achieve our required specifications. We have prepared circular DNA constructs (100bp, 106bp, and 108bp) sustaining comparable magnitudes of bending stress and varying degrees of torsional stress (which arises when linear DNA molecules of a non-integral number of helical turns are circularized). Using cryo-electron microscopy (cryo-EM) combined with 3-D image reconstruction, we have been able to quantitatively characterize the structural details at the molecular level of the topological effects of torsional stress within these minicircle constructs. We have observed the three species of minicircles under conditions of both weak and mild electrostatic repulsion, and measured the observed distributions of curvature (indicative of kink formation) and writhe (reflective of torsional stress). Despite the significant torsional stress sustained within the most highly stressed construct, all three are roughly planar, though the writhe and curvature distributions do depart significantly from theoretically predicted values. We are attempting to resolve the discrepancies between theoretical expectations and our observed experimental data using Brownian dynamics simulations of DNA minicircles sustaining varying degrees of torsional stress. We expect that this work will begin to define the behavior of highly stressed DNA at biologically relevant scales, and will broaden our understanding of how sub-persistence length DNA responds to mechanical stress.

2417-Pos

Measurement of the Elastic Energy of Sharply Bent Ds DNA

Hao Qu, **Yong Wang**, Chiao-Yu Tseng, Giovanni Zocchi.
UCLA, Los Angeles, CA, USA.

We present measurements of the elastic energy of short (30 bp), sharply bent, ds DNA molecules. The measurements are obtained by two independent methods: one is based on the monomer-dimer equilibrium of an appropriate configuration where the elastic energy stored in the bent strands drives dimer formation; the other is based on melting curves analysis. We find that, for example, the elastic energy of a sharply bent 30 bp double stranded DNA molecule with a nick at the center does not exceed 10 kBT.

2418-Pos

Visualizing and Quantifying the Energy Landscape during DNA Overstretching

Peter Gross[1], Niels Laurens[1], Lene B. Oddershede[2], Ulrich Bockelmann[3], Erwin J.G. Peterman[1], Gijs J.L. Wuite[1].
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]Niels Bohr Institute, Copenhagen, Denmark, [3]ESPCI, Paris, France.

DNA undergoes a structural transition at a tension of 65 pN, were the polymer gains 70% of its contour length. The molecular basis of this overstretching transition has been elucidated using a combination of fluorescence microscopy and optical tweezers: At a tension of 65 pN, the DNA undergoes a nucleation-limited force-induced melting transition, in which the DNA strands gradually fray from the DNA's extremities during progression of overstretching [1].
Here we demonstrate that inhibition of this fraying process from one of the two DNA ends leads to a single deterministic melting front, which allows us to correlate the force signal in the overstretching plateau to the melted sequence.
We show that the propagation of the melting front progresses in bursts involving cooperative unbinding of multiple base-pairs. We furthermore proof that this burst-wise melting is an equilibrium process that is completely determined by the DNA sequence. Applying an equilibrium molecular stick-slip theory, we obtain a good agreement in both the force at which the DNA molecule starts to denature and the location of the individual melting bursts. We demonstrate that this theory, with the underlying DNA sequence as input, is able to predict the force-induced melting behavior.
Furthermore, we explore individual melting bursts by monitoring the force level of a DNA close to a melting event, and observe a bistability between two levels of the melting process. The time scale between the melting-reannealing events provides us with insight into the underlying local energy landscape close to a melting burst.
[1] van Mameren et al., Unraveling the structure of DNA during overstretching using multicolor, single-molecule fluorescence imaging, PNAS (in press) 2009

2419-Pos

Torsional Studies of DNA Denaturation using Angular Optical Trapping

Maxim Y. Sheinin[1], Scott Forth[1,2], Michelle D. Wang[1,2].
[1]Department of Physics, Cornell University, Ithaca, NY, USA, [2]Howard Hughes Medical Institute, Cornell University, Ithaca, NY, USA.

Torque-induced separation of duplex DNA strands plays an important role in a wide variety of cellular processes, such as transcription, DNA replication,

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.

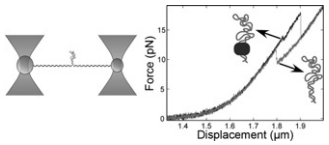
Furthermore, dsRNA has important roles within biology, in its own right; not least among these, is that dsRNA is the central player in the gene-silencing pathway mediated through small interfering RNAs (siRNAs). The novel dsRNA substrates we have created, now pave the way for a more detailed understanding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