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,