microscopy analysis. We evaluate our experimental data correlating topological changes with specific translocation event morphologies using all-atom Molecular Dynamics simulations. Our results provide new fundamental insight into characterizing DNA topology, and has important implications for anti-cancer drug treatment and design.

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1766-Plat
Mechanical Properties and Strand Invasion of Duplex Telomere DNA Probed using Magnetic Tweezers
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Telomeres are specialized chromatin structures that protect chromosome ends from nucleolytic processing by DNA repair machinery. The foundation of human telomere structure consists of a long array of tandem duplex DNA sequences (TTAGGG) and terminates with a single-stranded 3' end. To protect the chromosome end, telomeres are thought to adopt a lariat structure known as a telomere-loop (T-loop)1. T-loops are stabilized by DNA displacement loops (D-loops) generated by the invasion of a single-stranded telomeric DNA tail into an adjacent region of duplex telomere. Recent studies suggest that telomere-associated proteins promote strand invasion during telomeric D-loop formation through the application of torque to the DNA2. Although the molecular mechanism of T-loop formation has been described using biochemical approaches, the torque response and internal structural equilibrium of duplex telomeric DNA are not well characterized. To probe the mechanical properties of duplex telomeric DNA, we developed a magnetic tweezers assay to detect the response of single telomeric DNA molecules to precisely applied degrees of tension and torque. Rotation-extension curves under varying tension demonstrate that the repetitive telomere DNA sequence is more refractory to torque-induced denaturation than a non-telomeric control molecule of comparable GC content. In addition, force-extension analysis of negatively supercoiled telomeric DNA in the presence of different counter-ions (K^+ vs. Li^+), reveals that transient torque-induced denaturation of duplex telomeric DNA promotes a structural transition into stable DNA G-quadruplexes. Lastly, using a single molecule DNA topology-based assay, we directly monitor the torque-dependent invasion of single stranded telomere DNA primers into duplex telomeric DNA tethers. Our results provide insight into the molecular mechanisms of telomere-associated proteins and enzymes during structural remodeling of telomeres.

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1767-Plat
High-Throughput Quantification of the Impact of Different Osmolytes on the Thermal Stability of DNA
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Small molecules (osmolytes) are known to either stabilize or destabilize proteins/nucleotides depending on the concentrations and/or solvent conditions. The presence of different molecules and ions in the surrounding medium affects the stability of DNA in solution. In this work, we have developed a High-Throughput method for quantifying the energetic impact of addition of various osmolytes on short DNA duplexes. Six 19-base pair, non-self-complementary duplex DNA oligomers along with a 16-base pair control duplex DNA, having varied GC-content (ranging from 16% to 79%), nearest neighbors and end sequences were used. We sampled thirteen different osmolytes that are common in humans and throughout nature by covering different chemical classes including, sugars, polyols, amino acids, and methylamines. Varying concentrations of these osmolytes (from 0.5 M up to 3.0 M) were examined for their effects on these duplexes. Experiments were performed in 384-well plates that were prepared using a robotic device, which was calibrated for the correct dispense volume for different components of the plate. Temperature-induced melting transitions monitored by fluorescence were measured for these duplexes and the Tm values along with the m-values and melting transition enthalpies were determined. Conventional approaches, including Circular Dichroism (CD) were used to verify the thermodynamic parameters. Osmolytes had varied effects on DNA stability, and the (de)stabilizing effect does not necessarily correlate with their effects on proteins. The m-values can drastically depend on the GC-content.

1768-Plat
Real Time Transposable Element Dynamics
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Transposable elements are mobile genetic elements that are capable of self-catalyzed excision or copying from their host's genome, followed by integration back into another location within the genome. Transposable elements increase in number over time as a result of this activity, and as a consequence can make up a substantial portion of the host's genome as ''junk DNA''; both active and dormant transposable elements make up at least 45% of the human genome, and up to 85% of the maize genome. Additionally, the unpredictable reintegration of transposable elements into coding or control regions of the host's genome can have dramatic effects on gene expression, and, as a result of this inherently mutagenic nature, transposable elements are thought to be a major source of genome plasticity driving evolution and are implicated as the direct causative agents of many human diseases, including hemophilia, porphyria, severe combined immunodeficiency, muscular dystrophy, and breast and colon cancers.
Despite their ubiquity and potential importance, very little is currently known about the dynamics of transposon propagation through genomes, and their contribution to evolution is inferred from comparative analyses of the genome sequences of related organisms. In this talk I will describe methods developed by my lab employing fluorescent microscopy, microfluidic, and molecular biology techniques to allow the direct visualization of transposable element activity in single cells and in real time. The proposed experimental system is extensible to all types of transposable elements and all cell types, from bacteria to human.

1769-Plat
Effect of Methylation on the Nanomechanics of Double-Stranded DNA
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In its physiological environment DNA is constantly exposed to mechanical stress. The nanomechanical properties of DNA influence not only its response to stress but also its interaction with proteins. Despite its crucial role in epigenetics, little is known about how methylation affects the nanomechanical properties of DNA. To investigate the impact of methylation on DNA nanomechanics, here we manipulated single molecules of chemically or enzymatically methylated DNA and compared their properties with those of non-methylated DNA. As a model we used a 3312-base-pair long sequence of lambda-phage DNA that met the criteria of a CpG island. Chemically methylated DNA was prepared with PCR containing 5-methyl-CTP in the reaction mixture. For enzymatic methylation the M.Sss.I methyltransferase was used. Single DNA molecules were mechanically manipulated with force-measuring optical tweezers in repeated stretch-relaxation cycles. Surface-adsorbed DNA molecules were studied by using atomic force microscopy (AFM). We found that the molecular contour length, bending rigidity and intrinsic stiffness were decreased in methylated DNA, pointing at structural and nanomechanical alterations. Furthermore, the cooperative overstretch transition was significantly longer in the methylated form of the molecule, suggesting that the dynamics of intramolecular rearrangements were also affected. AFM measurements of DNA molecules adsorbed to mica surface substantiated the significant reduction of molecular contour length in methylated DNA. By contrast, the apparent bending rigidity of the surface-adsorbed methylated DNA was increased, which is most likely caused by interactions between DNA and the mica surface. In sum, methylation leads to an axial compaction of the dsDNA structure, an increase in bending flexibility in the low-force regime and an increase in axial compliance at higher forces (>20pN). Conceivably, modulation of DNA structure and nanomechanics caused by methylation leads to a complex control of structural accessibility and association kinetics of DNA-binding proteins.

1770-Plat
Thermodynamics for the Interaction of PEG-PLL Copolymers with DNA
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One focus of our research is to select polycations to deliver oligonucleotides into the cell for the control of gene expression. In this work, we report on the interaction of poly(ethylene glycol)-b-poly-L-lysine (PEG-PLL) copolymer with a variety of DNA molecules. Specifically, three PEG-PLL copolymers with similar PEG segment but different length of the PLL chains were used to interact with DNA duplexes as a function of duplex length. A combination

of spectroscopic and calorimetric techniques was used to investigate the unfolding of both DNA molecules and polycation-DNA complexes, and to determine their thermodynamic binding profiles. The resulting polycation-DNA complexes were stable in aqueous solution at room temperature. The binding of each copolymer to DNA stabilized the helix-coil transition of all DNA molecules, yielding binding affinities of ~104 M-1, which were lowered by the increase in salt concentration. However, binding affinities of 105 were obtained with the ethhidium bromide displacement essay. Isothermal titration calorimetric experiments yielded negligible heats of interaction. Therefore, the favorable formation of the copolymer-DNA complexes is entropy driven which was rationalized in terms of the release of both counterions and water molecules upon complex formation. In summary, polycation binding to DNA was found to be electrostatic in nature, i.e., the positively charged lysine groups formed ion pairs with the negatively charged phosphate groups of DNA. Supported by Grant MCB-1122029 from the National Science Foundation.

1771-Plat

Correlating Drug Binding Affinities with Base Pair Opening Rates in DNA

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The cyclic AMP responsive element (Cre) is a highly conserved stretch of DNA that is involved in the activation of gene transcription. Our group has studied the drug binding of a fluorescent derivative of a DNA intercalating anti-cancer drug, 7-amino actinomycin D (7-AMD), to the Cre sequence in different sequence contexts. We initially analyzed the DNA backbone conformation of Cre samples with varying flanking sequences and correlated these values to the binding affinities of 7-AMD. These studies revealed several anomalies that suggest that the conformation described by BI/BII content of the DNA backbone is, at most, only partially responsible for the 7-AMD binding affinity to the Cre sequence. This result is not surprising as 7-AMD has a conjugated ring structure in addition to its peptidyl side chains which interact with the backbone. As a result, we began studying DNA base pair opening rates and correlating these with 7-AMD binding affinities to account for the intercalation of 7-AMD into the Cre sequence. DNA base pair opening rates were determined by tracking imino proton exchange via NMR spectroscopy in the presence of a varying concentration of base catalyst. In this study, both two-dimensional NOESY and one-dimensional H^1 NMR spectroscopy are used to track the change in line widths of the imino protons of the central Cre binding site for five sequences with varying flanking sequences. Trends in opening rates reveal that sequences with strong 7-AMD binding feature slower base pair opening dynamics than sequences with weaker 7-AMD binding. These results suggest that local base stacking is important for 7-AMD binding, a hypothesis we are currently investigating with UV spectroscopy.

Platform: Exocytosis, Endocytosis, and Membrane Fusion

1772-Plat

Microtubule Motors Drive Plasma Membrane Tubulation in Clathrin-Independent Endocytosis

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How the plasma membrane is bent to accommodate clathrin-independent endocytosis is poorly understood. Recent studies suggest the exogenous clathrin independent cargo molecules Shiga toxin and cholera toxin induce the negative membrane curvature required for endocytic uptake by binding and crosslinking multiple copies of their glycosphingolipid receptors on the plasma membrane. But it remains unclear if toxin-induced sphingolipid crosslinking provides sufficient mechanical force for deforming the plasma membrane, or if host cell factors also contribute to this process. To test this, we imaged the uptake of cholera toxin B-subunit into surface-attached tubular invaginations in live cells. We found that a cholera toxin mutant that binds to only one glycosphingolipid receptor accumulates in tubules, and that toxin binding is entirely dispensable for membrane tubulations to form. Unexpectedly, the driving force for tubule extension was found to be supplied by the combination of microtubules, dynein, and dynactin, thus defining a novel mechanism for generation or extension of membrane curvature during endocytic uptake at the plasma membrane.

1773-Plat

High-Speed Atomic Force Microscopy of ESCRT Protein Assembly

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The endosomal sorting complex required for transport (ESCRT) mediates membrane remodelling in cells. When ESCRT oligomerize, it is able to bud the membrane forming constriction necks that will break resulting in vesicular bodies or the viral envelope, to name a few of its implications. So far, relatively little is known about the molecular fine structure and less about the dynamics of ESCRT assembly, essential for our understanding how it deforms and cleaves the membrane.

In this work, we used high-speed atomic force microscopy (HS-AFM) to study the ESCRT machinery, in particular the ESCRT-III complex, Snf7. HS-AFM allows simultaneous observation of structure, dynamics and function of biological assemblies, with nanometer spatial and sub-second temporal resolution. We show HS-AFM movies of the Snf7 complex formation and its dynamics from filament to the maturated circular assembly around the membrane constriction site. We observe interfilament dynamics that provide a basis for a mechanistic explanation how the machinery creates tension for membrane fission by a buckling mechanism.

1774-Plat

Mechanisms of Membrane Shaping by Peripheral Proteins

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Membrane curvature has developed into a forefront of membrane biophysics. Numerous proteins involved in membrane curvature sensing and membrane curvature generation have recently been discovered, and the structure of these proteins and their multimeric complexes is increasingly well-understood. Substantially less understood, however, are thermodynamic and kinetic aspects and the detailed mechanisms of how these proteins interact with membranes in a curvature-dependent manner. New experimental approaches need to be combined with established techniques to be able to fill in these missing details. Here we use model membrane systems in combination with a variety of biophysical techniques to characterize mechanistic aspects of the function of peripheral proteins such as BAR domains, ENTH domains, and synucleins. This includes a characterization of membrane curvature sensing and curvature generation. We also establish kinetic and thermodynamic aspects of BAR protein dimerization in solution, and investigate kinetic aspects of membrane binding. We present two new approaches to investigate membrane shape instabilities leading to stable membrane curvature. We demonstrate that membrane shape instabilities can be controlled by factors such as protein binding, lateral membrane tension, lipid shape and asymmetric bilayer distribution, and macromolecular crowding on the membrane.

Our findings are relevant to the mechanistic understanding of membrane trafficking phenomena, including endocytosis.

1775-Plat

Role of Hemagglutinin Palmitoylation in Assembly and Fusion of Influenza Virus-Like Particles

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Influenza A virus is a major human pathogen causing annual epidemics and occasional pandemics. Hemagglutinin (HA), the influenza virus fusion protein, contains on its cytoplasmic tail three conserved cysteins which are palmitoylated. Contradictory data have been reported regarding the role of HA palmitoylation in either membrane fusion or virion assembly. Here we analyzed the role of HA palmitoylation on assembly and fusion of influenza virus-like particles (VLPs) by using fusion assay, cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET). VLP assembly, release, morphology, as well as glycoprotein spacing on the surface of the VLP, were not affected by mutation of all three cysteins. However, using both cell-cell and VLP-cell fusion assays we found that palmitoylation plays a role in fusion pore enlargement. We tested HA from three different influenza strains (H2 (A/Japan/305/57), H3 (A/Aichi/2/68), H3 (A/Udorn/72)). In all cases HA depalmitoylation impaired pore enlargement, suggesting that the role of palmitoylation in