model which accounts for DNA bending, torsion, electrostatics, and hydrodynamics. Our simulations are designed to parallel experiments and permit direct comparisons to the experiments.

1415-Pos Board B145

Persistence Length of Single Stranded DNA: Effect of Length, Sequence and Surface

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The knowledge of the DNA persistence length indicates the chain's flexibility which is related to the possible final folded state of DNA and provides the means for understanding DNA's structure in solution and on surfaces. The persistence length of ssDNA depends on the rigidity of the backbone and an intra-chain repulsion due to negatively charged backbone. In order to determine the contributions of rigidity and electrostatics on persistence length of ssDNA we conducted series of molecular dynamics simulations with regular and neutralized DNA of various length and sequences. We found that persistence length is largely determined by the flexibility of the backbone. However the electrostatic contribution to the persistence length is sequence dependent due to differences in base stacking and hydrogen bonding network.

To investigate the effect of surface on the structure and dynamics of single stranded DNA (ssDNA) we performed molecular dynamics simulations of surface constrained ssDNA. We observed that surface grafting of ssDNA significantly changes its folding pathway, pi-pi stacking interaction, persistence length, and end to end distance when compared to free ssDNA. Moreover, we found that the number of bases and sequence play an important role in structure and dynamics of ssDNA constrained on the surface. Our research provided atomistic understanding of dynamics and conformational changes of single stranded DNA under various conditions; the length and sequence dependence as well as the effect of surface immobilization.

1416-Pos Board B146

Kinetics of DNA Threading Intercalation by a Rigid Ruthenium Complex Dimer

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Compounds that can intercalate DNA by threading between melted bases serve as a model of therapeutic drugs for malignant tumors. An essential property of effective antitumor activity requires a drug candidate to exhibit a slow dissociation rate from the intercalation state of DNA-drug complex. In particular, previous bulk experiments reported a very slow dissociation rate for the rigid Ruthenium complex dimer ΔΔ-P (Δ,Δ-[μ-(11,11'-bidppz)$(phen)_4Ru_2]^{4+}$). We examined the kinetics of ΔΔ-P threading intercalation into an individual λ-DNA molecule held between a micropipette and an optical trap over a concentration range of 2-100 nM. DNA extensions due to threading intercalation are measured at several concentrations for constant stretching forces of 10-60 pN. As a result, fractional binding is determined as a function of force and concentration from the time-dependent approach to equilibrium extension, which yields equilibrium binding affinity. We also obtain the force and concentration-dependent on and off rates for the threading reaction. Furthermore, these measurements allow us to obtain the force-dependent and zero force binding affinity. By fitting these rates to the expected exponential dependence on force, we are able to extract the equilibrium change in DNA extension due to a single intercalation event as well as the distance to the transition state from the bound and unbound equilibrium states. These results show that the DNA length must increase significantly both for association and dissociation of the ligand, which explains the extremely slow binding kinetics.

1417-Pos Board B147

Single Molecule Force Measurements of DNA and RNA Hairpin Structures

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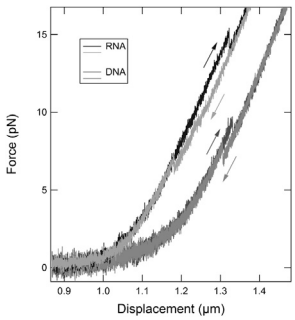
DNA and RNA hairpins contribute to fundamental mechanisms of molecular biology, for instance to the regulation of transcription and translation. Moreover, the hairpin secondary structure is a model system for studying the dynamic assembly of nucleic acid structures. Using a high-precision dual-trap optical tweezers setup, we performed a quantitative investigation of the folding dynamics of hairpins under mechanical load.

A hairpin structure is composed of a double-helical stem and a single-stranded loop. Two different hairpins were studied, each one as a DNA and as an RNA molecule. They have the same stem of 13 basepairs, but exhibit loops of 10 and 18 nucleotides respectively. Significant differences are observed, not only between the two hairpins but also between DNA and RNA. As illustrated in the figure, hysteresis between unfolding and refolding curves is much stronger for RNA than for DNA. For narrow-loop hairpins, flipping between folded and unfolded states is observed for DNA and RNA. The wide-loop hairpins, however, show flips only for DNA. Altogether, our results show that the RNA molecule is more easily driven out-of-equilibrium by an external perturbation than DNA.



1418-Pos Board B148

DNA-Intercalation Kinetics Elucidated by Single-Dye Fluorescence Microscopy and Force Spectroscopy

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Intercalating dyes are versatile molecules for fluorescence visualization of double-stranded DNA. Despite their widespread use in single-molecule and bulk biochemical assays, a complete mechanistic and kinetic insight in their interaction with DNA is lacking, which hampers optimization of biological assays using these molecules. Here, we study intercalation of double-stranded DNA by visualizing single DNA-intercalation events in real-time using fluorescence microscopy and controlling DNA tension with optical tweezers. This allows, for the first time, to fully quantify the affinity and kinetics of the mono-intercalators SYTOX Orange, SYTOX Green, SYBR Gold, and YO-PRO-1, and of the bis-intercalators YOYO-1, and POPO-3. We show that all these intercalators obey single-step, first-order DNA-binding kinetics and that their affinity for DNA is governed by an off-rate that decreases exponentially with DNA tension. Remarkably, the DNA tension alters the DNA-intercalator interaction time by two to four orders of magnitude. In addition to this strong tension dependence, the affinity for DNA also depends strongly on the intercalator species and on the ionic strength of the buffer. These new fundamental insights provide opportunities to selectively perform experiments either in or far out-of-equilibrium. We demonstrate that this kinetic control can be exploited to optimize biological assays based on intercalation: it allows minimizing the perturbation that intercalators impose on the dynamics of structural transitions during DNA overstretching and on processive enzymatic reactions such as DNA replication.

1419-Pos Board B149

Simultaneous DNA Stretching and Intercalation in Continuous Elongational Flow

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Fluorescent microscopic observation of DNA stretching in homogenous elongational flow has previously been used to characterize biophysical properties of the polymer. Typically, individual molecules are trapped at the stagnation point of opposed fluidic flows in a cross-slot microfluidic structure. Such observations intrinsically have a low throughput of analyzable molecules. We present a continuous-flow microfluidic funnel that replicates the intramolecular tension distribution established in cross-slot DNA stretching experiments, but achieves greater than 10 megabase per second analyzable DNA throughput.

Intercalating fluorescent dyes are required for single-molecule visualization of stretched DNA. DNA elasticity under tension however is affected by bound intercalator. Careful experimental control of the relative concentration of intercalator and DNA is therefore required for reproducible DNA stretching. In the presented device, intercalator is introduced using convergent sheathing flows, which center DNA in the microfunnel over a series of confocal laser excitation spots. The intercalation reaction therefore proceeds one molecule of DNA at a time, thus eliminating dependence on DNA concentration. Using this experimental platform, we demonstrate differing effects on DNA elasticity for the monomeric dye POPRO-1 and its bis-intercalating analogue POPO-1. We also characterize the effect of intramolecular tension on the orientation of intercalating dye in its DNA binding site using single-molecule fluorescence anisotropy.