2432-Pos
A Theoretical Description of DNA Plectonemes under Tension
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Twisting a DNA molecule held under constant tension is accompanied by a transition from a linear to a plectonemic DNA configuration, in which part of the applied twist is absorbed in a superhelical structure. This is seen as a linear shortening of the DNA length with added turns after the transition. So far no theoretical description exists, which consistently describes the slope of the supercoiling curves as well as the torque in the plectonemic regime and its dependency on the applied force and the monovalent ion concentration in solution. Here, we present a simple model, in which the DNA is treated as a semiflexible rod. The energy of the plectonemic structure is calculated considering DNA bending, applied tension and electrostatic repulsion between the DNA strands but excluding fluctuations. We compare the predictions of our simple static theory with experimental supercoiling data, recorded with magnetic tweezers. We obtain an excellent agreement for the supercoiling slopes and the torque as function of force and monovalent ion concentration only if a reduced DNA charge is taken into account. We verify our theory using Monte-Carlo simulations, in which the same energetic terms are used. Surprisingly, the simple static model describes experimental data much better than more sophisticated models considering fluctuations, which considerably overestimate the torque of the plectonemic phase.

2433-Pos
Monte Carlo Simulation of Supercoiled DNA at Buckling Transition
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Recent studies of high resolution single molecule experiments yielded detailed information of DNA supercoiling under applied tension. Here we use Monte Carlo simulations with a coarse-grained DNA model to improve the understanding of these data. To reproduce experimental conditions, stretching, bending, twisting and electrostatic potentials were explicitly considered in the computer model.
As in single-molecule experiments with magnetic tweezers, we carry out simulations for different applied forces and ionic strengths over a large range of applied supercoils. The simulations reproduce well the experimentally observed behavior: While initially the molecule extension remains almost constant upon twisting, a linear decrease in extension with added twist is observed, once a critical buckling torque is reached. At higher ionic strength this is caused by the formation of a superhelical, i.e. plectonemic, structure. At these conditions the buckling transition between stretched and plectonemic DNA is accompanied by a abrupt DNA length decrease. At low ionic strength however, the buckling phase vanishes and the formation of multiple loose DNA loops is preferred over a superhelical structure. Interestingly under these conditions, the torque does not remain constant anymore with added turns.
Beyond an overall qualitative agreement, the MC simulations reproduce quantitatively most of the experimental parameters, if the interaction potentials are appropriately chosen. This includes the slope and torque of the linear decrease after buckling but also the jump size and the torque change during abrupt buckling. The computer model allows thereby new insights into the torsional and electrostatic behavior of supercoiled DNA. Further details not directly accessible in experiments like plectoneme geometry or singular energy distributions can easily be derived.

2434-Pos
Anharmonic Torsional Stiffness of DNA Revealed under Small External Torques
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DNA supercoiling plays an important role in a variety of cellular processes by forcing the double helix to bend, fold, and wrap around proteic particles. The torsional stress related with supercoiling can be also involved in gene regulation via local modulations of DNA structure and dynamics. This idea is often invoked in the literature, but the physical mechanisms by which global supercoiling can act locally are unknown.
We tried to get an insight in this issue by using all-atom MD simulations. Recent methodological advances improved the accuracy of the torsional persistence length (l_t) measured from MD data and also made possible simulations with steady torsional stress applied to short stretches of DNA. The steady stress emulates local conditions of a short fragment under global supercoiling. The small DNA length reduces the computational load and makes possible extensive sampling and statistical convergence. We could measure linear elastic responses as well as elastic parameters of DNA under torsional stress corresponding to physiological supercoiling.
We found that small static untwisting significantly reduces l_t of GC-alternating DNA. For the AT-alternating sequence a smaller effect of an opposite sign is observed. For these two sequences, the l_t values are similar under zero stress, but diverge with untwisting. The effect is traced to sequence-specific asymmetry of local torsional fluctuations. Analysis of other sequences suggests that this property is rare and probably undetectable in long random DNA. However, in short stretches of some specific sequences, small natural modulations of supercoiling can significantly alter the probabilities of twisting fluctuations by making the double helix locally softer or stiffer, which gives a simple possibility of gene regulation. Our results also have interesting implications for the role of local DNA twisting in complexes with some transcription factors.

2435-Pos
DNA Melting Induced by Temperature and Mechanical Strain
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The polyelectrolyte nature of DNA greatly complicates the reaction coordinates for both hybridization and melting. Here we consider mechanism of the melting of DNA by thermal and mechanical means. To study the melting transition of DNA, we used molecular dynamics simulations of a homogeneous 12-basepair DNA d(A12)•d(T12) with explicit water and ions at 400 K. The trajectories were analyzed with principal component analysis and revealed various processes which occurred on different time scales. A multistep mechanism is proposed where the untwisting of the duplex coupled with the breakup of the ion atmosphere is determined to be the rate-determining step of the melting process. To complement this study, sequences of DNA in states of linking number from +10 to −10 were studied. The mechanical strain is intimately coupled to the ionic distributions which determines the relaxation mechanism. The two mechanisms are remarkably different.

2436-Pos
A Molecular Dynamics Study of DNA Bending in the IHF-DNA Complex
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The complex of the protein Integration Host Factor (IHF) with oligomeric DNA results in a structure in which the DNA is bent by nearly 180 degrees, referred to by the crystallographers reporting on the structure as a "U-turn". This is a highly unusual form of duplex DNA, and the nature of how the structure forms is a question of current research interest. This project involves using molecular dynamics (MD) computer simulation to study the dynamics involved in the bending process, in particular the extent to which the U-shaped structure is pre-programmed into the DNA sequence or induced by the IHF-DNA interaction. The protein-DNA crystal structure consists of a 35-mer B-strand singly-nicked DNA sequence and the IHF protein unit of 193 amino acid residues (PDB #1IHF). Additionally, an MD simulation was run on a canonical B form DNA oligomer of identical sequence to examine the resulting convergence of the two starting DNA structures. The MD was performed using the AMBER suite of programs as implemented on the Wesleyan PC cluster. The molecular graphics and animations were carried out using the programs VMD and SwissPDB DeepView. Simulations including water and counterions were performed on both the nicked and sealed versions of the U-turn DNA, the fabricated canonical B DNA, the free IHF protein, and the entire IHF-DNA complex.

2437-Pos
Understanding Protein-DNA Interactions through Dynamics
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Transcriptional regulation is a key factor in controlling proper cellular behavior. For this reason, so-called regulation networks (quantifying the molecular interactions controlling the transcription), have been heavily studied. One goal is to enrich these networks through in silico identification of DNA-binding proteins and their respective binding sites. Often such work assumes a specific distance between atoms as constituting an interaction and construct models based on this assumption. However, this ad hoc rule fails to account for many of the complexities that lie behind the physical nature of binding. We present a framework for studying these interactions in more realistic settings accounting for both overall energy and dynamics of protein-DNA complex. We demonstrate that short molecular dynamics simulations better characterize biomolecular interactions and that a better definition of interactions improves the prediction of protein-DNA docking. Specifically, interacting residues are identified through the analysis of MD energy functions and results are

compared with published experimental results. Further, we show how our novel definition of DNA-binding can be used for constructing improved machine learning classifiers for automatic identification of DNA-binding residues.

RNA Folding

2438-Pos
Probing the Dynamics of the P1 Helix within the Tetrahymena Group I Intron
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RNA conformational transformations are integral to RNA's biological functions. Further, structured RNA molecules exist as a series of dynamic intermediates in the course of folding or complexation with proteins. Thus, an understanding of RNA folding and function will require deep and incisive understanding of its dynamic behavior. However, existing tools to investigate RNA dynamics are limited. Here we introduce a powerful fluorescence polarization anisotropy approach that utilizes a rare base analog that retains substantial fluorescence when incorporated into helices. We show that 6-methylisoxanthopterin (6-MI) can be used to follow the nanosecond dynamics of individual helices. We then use 6-MI to probe the dynamics of an individual helix, referred to as P1, within the 400nt Tetrahymena group I ribozyme. Comparisons of the dynamics of the P1 helix in wild type and mutant ribozymes and in model constructs reveal a highly immobilized docked state of the P1 helix, as expected, and a relatively mobile 'open complex' or undocked state. This latter result rules out a model in which slow docking of the P1 helix into its cognate tertiary interactions arises from a stable alternatively docked conformer. The results are consistent with a model in which stacking and tertiary interactions of the A3 tether connecting the P1 helix to the body of the ribozyme limit P1 mobility and slow its docking, and this model is supported by cross-linking results. The ability to isolate the nanosecond motions of individual helices within complex RNAs and RNA/protein complexes will be valuable in distinguishing between functional models and in discerning the fundamental behavior of important biological species.

2439-Pos
Analysis of RNA Hairpin Folding by Dual Beam Fluorescence Fluctuation Spectroscopy
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Hairpin formation is one of the central problems of nucleic acid folding. Experimental and theoretical results from several laboratories indicate that in many cases formation of RNA hairpin cannot be treated as a simple two-state process. We use fluorescence fluctuation spectroscopy (FFS) to characterize folding kinetics of two RNA hairpins (CGGUUUCCCUCCCACCUUUGCCG and CGGUUUUUUUUUUUUUUUGCCG) each labeled with a fluorophore and a quencher (TAMRA and dabcyl) on the 3' and 5' end respectively. FFS is unique in its ability to measure relaxation rates at equilibrium, and coupling this technique to flow allows for simultaneous detection of the end-to-end contact and diffusion coefficient of the molecules. The two hairpins studied here have same stem but different loop sequences, and display different folding kinetics as a function of K^+ and Mg^{2+} ions. The results are interpreted in light of the calculated free energy landscapes.

2440-Pos
The Role of Bulges and Hinges in RNA Folding
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Long-range tertiary interactions govern RNA structural assembly, which is a critical step toward RNA biological functionality. Thereby, a universal strategy has emerged for global conformational change; flexible junctions enable unpaired nucleotides to act as beacons between helical regions. Bulges, for example, are versatile secondary structural elements implicated in helix recognition and packing. The P4-P6 domain of the *Tetrahymena* ribozyme utilizes this folding strategy by hinging to form two inter-helical tertiary contacts, the adenine-rich (A-rich) bulge and the tetraloop-tetraloop receptor interactions. To explore the kinetic and thermodynamic properties of tertiary contact formation, we probe the P4-P6 domain hinging and ribose zippering that forms the A-rich bulge interaction using single-molecule FRET methods. We obtain the docking and undocking rates of the A-rich bulge and P4 helix as function of cation concentration and temperature. Docking is accelerated and undocking decelerated by Mg^{2+}. In spite of rapid docking at high $[Mg^{2+}]$ ($k_{dock} = 20 \pm 2\ s^{-1}$), the A-rich bulge interaction is only marginally stable ($K_{dock} = 1.2 \pm 0.1$). These results support that the role of the A-rich bulge is to kinetically direct P4-P6 domain folding while thermodynamic stability is added through the tetraloop-receptor interaction. Formation of the A-rich bulge contact shows specificity for divalent cations, with a preference for Mg^{2+} as anticipated from the Mg^{2+} coordination observed in structural data. A significant kinetic heterogeneity is characterized; only 50% of the molecules exhibit efficient folding at high $[Mg^{2+}]$. Mutations of the A-rich bulge construct reveal a crucial role of the P4-P6 secondary architecture in enabling the A-rich bulge contact.

2441-Pos
Single Molecule FRET Studies of RNA Tertiary Folding: Elucidating the Thermodynamic and Kinetic Role of Na+ Cations
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Understanding the kinetics and thermodynamics of the stabilizing interactions in non-coding RNA provides crucial information about their structural dynamics and ultimately, their biological function. The use of single-molecule FRET methods allows us to investigate the real-time folding and unfolding of an isolated GAAA tetraloop-receptor interaction in the *Tetrahymena* ribozyme. Cation-dependent folding studies of this ubiquitous interaction show that increasing concentrations of Na^+ significantly increase the rate constant for docking and slightly decrease the rate constant for undocking. We examine the temperature-dependence of this Na^+-induced folding in order to determine the thermodynamic parameters associated with the folding and unfolding processes. At 150 mM Na^+ the folding process is exothermic ($\Delta H^\circ = -20$ kcal/mol) but with a significant entropic cost ($\Delta S^\circ = -67$ cal/mol K), leading to a near zero ΔG° at 298 K. Increasing concentrations of Na^+ dramatically increase both ΔH° and ΔS°, with the competition yielding a decrease in ΔG°. For example, by 600 mM Na^+ the folding process even becomes endothermic ($\Delta H^\circ = 4.3$ kcal/mol) and entropically rewarding ($\Delta S^\circ = 20$ cal/mol K), with the folding process now becoming slightly favorable ($\Delta G^\circ = -1.7$ kcal/mol). These results indicate that increasing Na^+ concentration favors folding by increasing entropic gains more than enthalpic losses, which leads to a more favorable folding free energy change. We propose a model that uses the competing roles of solvent and structure to explain these gains and losses.

2442-Pos
The Role of Helix Topology and Counterion Distributions in RNA Interactions
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RNA and DNA helices have the same charge, −2e/bp, but different helical structures. The 2'-OH present in RNA hinders duplex flexibility and promotes the A-form helix whereas the more malleable and polymorphic DNA duplexes prefer the B-form. Using a combination of experimental and computational approaches, we show that the topology of the A-form helix alters the spatial distribution of counterions and is essential in promoting the charge screening efficiency of RNA helices. Results from Anomalous Small-Angle X-ray Scattering (ASAXS) experiments suggest that monovalent and divalent cations are more closely localized to the RNA central axis due to A-form major groove penetration. This leads to very efficient change screening in RNA helices which has implications for ion-mediated RNA interactions in two important areas: RNA folding reactions and the design of short RNA helices for RNA interference applications.

2443-Pos
Temperature-Dependent Single-Molecule Fluorescence Measurements of RNA Motifs Melting
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Single-molecule studies have proven to be a powerful technique for studying folding and unfolding of molecules like proteins and nucleic acids. Population histograms of the Forster Resonance Energy Transfer (FRET) values of double-labeled molecules allow us to discern subpopulations within the ensemble which were previously inaccessible in bulk experiments. Typically, changing solution conditions like pH and ionic strength populate different conformational states allowing the kinetics of folding to be measured. However, temperature as a parameter has not been explored in single-molecule folding studies due to instrument limitations. We have developed and characterized a temperature-controlled single-molecule experimental setup with a range of up to 65C. We used our setup to measure the temperature-dependent melting of nucleic acids motifs like junctions and loops.