2966-Pos Board B13

Disruptions of the Regular Structure in DNA Minicircles

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DNA bending and torsional deformations, which often occur during its functioning inside the cell, can cause local disruptions of the regular helical structure. The disruptions created by negative torsional stress have been studied in detail, but those caused by bending stress have only been analyzed theoretically. To address the problem, we probed the structure of very small DNA circles, 63 - 105 bp in length, by single-strand-specific endonucleases. We determined that bending stress disrupts the regular helical structure when the radius of DNA curvature is smaller than 3.5 nm. The experimental data suggest that strong DNA bending initiates kink formation while preserving base pairing. To get quantitative information about the disruptions we developed a statistical-mechanical model of the disruption formations in DNA minicircles. The model, used in the computer simulation, specifies the disruptions by three parameters: DNA bend angle at the disruption θ_d, local DNA unwinding caused by the disruption formation, and the free energy associated with the disruption formation in unstressed double helix, G_d. We obtained a relationship between values of G_d and θ_d under which the theoretical results are compatible with the experimental data. The relationship suggests that the free energy of a base pair opening, which includes flipping out both bases, is significantly higher than the generally accepted value.

2967-Pos Board B14

Twist and Tension-mediated Elastic Coupling between DNA-Bending Proteins

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The mechanical properties of DNA are harnessed through bending, looping, and twisting by proteins that play critical roles in both reading and packaging the genome. Cooperative binding of such proteins is required to assemble transcription factor complexes in promoter regions and to condense DNA into higher-order chromatin structures. We study, using theoretical modeling, the effective interaction between DNA-bending proteins that arises from elastic stresses in the linker when the DNA is subject to tension. Our model for the cooperative effects of introducing multiple bends into DNA utilizes the worm-like chain formalism with twist resistance and external force. We find that the coupling free energy between two proteins exhibits damped oscillations with increasing distance between the binding sites, due to a preference for the proteins to align in an anti-parallel fashion along a DNA helix that resists twist. The oscillations are superimposed on an attractive potential arising from the bending deformation energy, which promotes aggregation to minimize the curvature of the linker DNA. These results allow us to calculate the mean first encounter time for bending proteins sliding along DNA and indicate an optimal applied tension for protein assembly. Furthermore, our calculations are used to study the unwrapping transitions of a dinucleosome under tension. Our results highlight the need to consider DNA twist even when no torsion is applied and the DNA ends are free to rotate. We demonstrate a variable-range oscillatory coupling between DNA-bending proteins that may provide a versatile potential mechanism for tension-mediated gene regulation.

2968-Pos Board B15

Dynamic Conformation Fluctuations of λ-Phage DNA in an Optical Trap

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Dynamic fluctuations in the conformation of λ-phage DNA have been observed in a fiber optic based light trap. The light force is used to confine a polystyrene bead (diameter, 0.5μm - 2.8μm) in three dimensions at a fixed distance from a flat surface coated with anti-digoxygenin antibody. The DNA is fixed to this bead through a biotin - streptavidin linkage on the 3′end, whereas a digoxygenin label is present on the opposing 5′. Through this simple assay, the dynamics of the free ends have been quantified for various times and separation distances. The dynamics have been observed using a high frame rate camera and the intercalating fluorophore, YOYO-1. In contrast to a conventional optical trap, this trap lacks a highly focused beam, greatly reducing localized heating and the associated risk to the biological specimen which could potentially cause error in this type of measurement.

2969-Pos Board B16

Simulations of Copper-1,10-Phenanthroline Complexes Binding the DNA

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Copper complexes of 1,10-phenanthroline (phen) are chemical nucleases employed as footprinting reagents for determining ligand binding sites. The cleavage activity of the parent complex, $Cu(phen)_2$, occurs according to the following mechanism: a) reduction of $Cu(phen)_2^{2+}$ to $Cu(phen)_2^{+}$; b) non-coordinative binding of $Cu(phen)_2^{+}$ to DNA; c) $Cu(phen)_2^{+}$ oxidation to $Cu(phen)_2^{2+}$ by H_2O_2, and formation of Cu-''oxo'' and/or Cu-''hydroxyl'' species; d) oxidative attack leading to DNA-cleavage.

However, the potential clinical use of the parent compound is mainly prevented by two drawbacks: i) the low binding constant of the second phenanthroline; ii) the modest sequence selective DNA cleavage.

To improve $Cu(phen)_2$ efficiency, Pitié *et al.* used a serinol bridge to link the two phen rings leading to Cu(2-Clip-phen) and Cu(3-Clip-phen) derivatives, which cleave the DNA 2 and 60 times more efficiently than $Cu(phen)_2$. To address the modest sequence selectivity, the amine group of the serinol bridge was functionalized with sequence specific DNA minor/major-groove binding ligands such as cisplatin- and distamycin-like compounds, leading to encouraging results.

In this work, a combination of theoretical methods, including DFT, Docking and Molecular Dynamics, was employed to i) characterize the DNA binding of these complexes and ii) to determine the origin of their diverse DNA-cleavage efficiency. Our simulations clearly revealed that several factors such as planarity of the ligand, better interaction with DNA and minor-groove fit, contribute to the enhanced efficiency of Cu(3-Clip-phen) compared to the other structurally similar complexes.

2970-Pos Board B17

P53 Induced Dna Bending

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Specific p53 binding-induced DNA bending and its underlying driving forces are crucial for the understanding of selective transcription activation. Diverse p53-response elements exist in the genome; however, it is not known how p53 specific binding induced DNA bending and DNA sequence influences the bending extent. Molecular dynamics simulations were performed on a series of p53 core domain tetramer-DNA complexes with various DNA sequences with difference in the central 4-base pairs of each half site to compare the DNA bending extent and pinpoint the underlying driving forces. The results show that the specific interactions between p53 dimer and DNA and between p53 dimers triggered intra- and inter-half sites DNA bending . The central 4 base pairs were important to the bending extent due to its variant flexibility. Specifically, the more flexible CATG containing DNA was able to maintain the specific interactions with the p53 including those from residues Arg280, Lys120 and Arg248 while those with CTAG that had low flexibility were less capable of maintaining the specific interactions . As a result, base pairings for the CATG sequence were stable throughout the simulation trajectory while those for the CTAG sequence was partially dissociated for part of the trajectory, which affected the stability of nearby Arg280-Gua base interactions. Thus, DNA bending was induced by the specific p53-DNA and p53 dimer-dimer interactions and the bending extent was dependent on DNA sequence that was correlated with its flexibility and ability to maintain specific interactions in bent conformations.

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2971-Pos Board B18

DNA Relaxation Dynamics in 1ID3 Yeast Nucleosome MD Simulation

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Nucleosomes are elemental structural units envolved in the formation of chromatin. Organization of chromatin has a profound effect on DNA transcription, replication, repair and recombination. Nucleosomes not only participate in the compaction of the genetic material but also regulate gene expression by controlling the accessibility of specific DNA binding sites to proteins. There are 33 crystal structures of nucleosomes currently available in the protein databank. Nucleosome typically consists of a 147 bp dsDNA wrapped around an octameric histone protein, (H2A.1-H2B.2)(H3-H4)2(H2A.1-H2B.2), in 1.65 left-handed superhelical turns. DNA interacts with histone octamer at 14 locations (every ~10 bp), forming a total of ~240 direct and indirect contacts and ~120 hydrogen bonds. Molecular Dynamics simulation is a useful technique for exploring dynamics at interaction

sites. We performed a 50 ns MD simulation of 1id3 yeast nucleosome and analyzed DNA motions in terms of calculated relaxation times and slopes of power law distributions for all 145 inter base pair steps. Relaxation times were found by fitting the autocorrelation functions of DNA helix parameters. Those for base pairs interacting with histone core could not be obtained due to the power law nature of dynamics at detected sites. Our results suggest that relaxation of DNA structure in the nucleosome is governed by two processess: 1) fast exponential decay (25 - 250 ps) followed by power law relaxation for base pairs that are more than 3.4 A away from the protein, and, 2) slow power law relaxation extending to 50 ns observed for base pairs interacting with histone subunit (less than 3.4 A away from protein). Proximity analysis confirms the presence of 14 histone-DNA interaction sites while autocorrelation and Fourier analysis proves to be useful for the studies of relaxation dynamics in nucleosomes.

2972-Pos Board B19

Side-by-side And End-to-end Attraction Of Double-stranded DNA

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Genomic DNA is densely packed inside the cell nucleus and viral capsids. Such close packing suggests that electrostatic repulsion between negatively charged DNA in the condensed state is balanced by counterion-induced attraction. Indeed, effective attraction between DNA in high-valence electrolytes has been experimentally demonstrated. Several theoretical models have been proposed to explain DNA attraction, however, specific microscopic mechanisms could not be unequivocally determined. Here, we report sub-microsecond all-atom molecular dynamics (MD) simulations of the effective force between double-stranded DNA in the side-by-side and end-to-end orientations. In a typical simulation, two DNA molecules were placed in an electrolyte solution a certain distance away from one another. An external harmonic potential was applied to keep the distance between the molecules constant, which allowed the effective mean force to be computed directly by averaging over 160 ns-long trajectories. We found that, in a side-by-side conformation, two DNA molecules can form a bond state in the presence of magnesium ions. In the bond state, DNA molecules contact each other via negatively charged phosphate groups, bridged by magnesium ions. For DNA in a monovalent electrolyte, the effective attractive force is too weak to induce DNA condensation in the presence of thermal fluctuations. In the end-to-end orientation, the attraction was found to take place regardless of the electrolyte concentration. The presence of a phosphate group at the 5′-ends of the fragments was found to direct DNA end-to-end self-assembly and produce bound states resembling a continuosus DNA molecule. Our simulations suggest that the end-to-end attraction, rather than being mediated by counterions, is likely caused by hydrophobic and the van der Waals interactions between terminal nucleobases of the fragments.

2973-Pos Board B20

Comparing Short dsRNA and dsDNA: Charge Screening Efficiency and Counterion Distribution

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Action of short double-stranded RNA (dsRNA) helices is a key component in the RNA interference mechanism. Since past theoretical and experimental work on nucleic acid electrostatics mainly focused on DNA, we conducted a comparative investigation of electrostatic effects in RNA and DNA. Using resonant (anomalous) and non-resonant small-angle x-ray scattering, we characterized the charge screening efficiency and counterion distribution around short (25bp) double-stranded DNA and RNA molecules of comparable sequence. Compared to dsDNA, we find that dsRNA molecules appear charge neutral on shorter length scales under conditions of lower bulk salt concentrations. The experimental results agree well with ion-size-corrected nonlinear Poisson-Boltzmann calculations. We propose that differences in electrostatic properties aid in selective recognition of different types of short nucleic acid helices by target binding partners.

2974-Pos Board B21

Structural Energetics of Two RNA-DNA Hybrids

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Intrinsic transcription terminators in bacteria are specific signals encoded in the base sequence of the DNA template. A canonical intrinsic terminator consists of a GC-rich dyadic sequence, followed by a track of five or more adenines in the template strand. The formation of a hairpin structure by the GC-rich dyadic sequence in the RNA transcript, and the low stability of dA-rU base pairs in the transcription RNA-DNA hybrid are believed to be the major contributors to the termination of transcription at these sites. In the present work, we have investigated two RNA-DNA hybrids corresponding to the GC-rich dyadic sequence in the tR2 terminator of phage λ. The stability of individual base pairs in these RNA-DNA hybrids was characterized and compared from measurements of the exchange rates of imino protons using nuclear magnetic resonance spectroscopy. The measurements also allowed determination of opening and closing rates for selected base pairs. The results indicate that a dA-rU base pair is destabilized relative to a dT-rA base pair in the same base sequence context. This destabilization is enhanced when two dA-rU base pairs are next to each other. The results also reveal that dG-rC base pairs have different stabilities from dC-rG base pairs. The magnitude of these differences depends on the base sequence context of the dG-rC/dC-rG base pairs, and on their proximity to dA-rU base pairs. (Supported by a grant from the NIH).

2975-Pos Board B22

Is the Formation of the Correct Nucleating Loop the Rate-Limiting Step in Hairpin Formation in ss-Polynucleotides?

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Numerous kinetics measurements on the formation of single-stranded (ss) DNA and RNA hairpin structures with ~4-20 nucleotides (nt) in the loop and ~5-8 base-pairs in the stem, indicate that the time required to form hairpins is ~10-500 microseconds. If ss-polynucleotide chain is treated as an ideal semiflexible polymer with a statistical segment length of ~4 nt, the theoretical estimate for the end-to-end contact time for an ~10-nt long chain is expected to be tens-of-nanoseconds. To explain this discrepancy in timescale, we proposed that the formation of the nucleating loop, prior to the zipping step, is slowed down as a result of transient trapping in misfolded conformations, with mis-paired base-pairs, non-native hydrogen bonding, or intrastrand stacking interactions in the unfolded state. Experimental measurements of end-to-end contact formation indicate that loop closure times for 4-nt poly(dT) loops are ~400 ns, and for 4-nt poly(dA) loops are ~8 microseconds, thus confirming that intrachain interactions slow down the configurational diffusion of the chain (Wang and Nau, J. Am. Chem. Soc. 2004, 126, 808). Interestingly, despite this evidence for intrachain interactions slowing down diffusion, the hairpin closing times for both ssDNA and RNA hairpins are found to scale with the length of the loop as $L^{2.2\text{-}2.6}$, in reasonable agreement with the scaling behavior expected for loop-closure of a semiflexible polymer.
Here, we present a kinetic zipper model that explicitly includes all misfolded microstates with non-native contacts, to describe the hairpin relaxation rates. The temperature and loop-size dependence for the relaxation rates is described in terms of two free parameters, the configurational diffusion coefficient that is relevant for the single-strand chain dynamics, and one parameter that characterizes the strength of non-native interactions prior to the formation of the nucleating loop.

2976-Pos Board B23

RNA Bending and Stabilization by Carbocyclic Sugars Constrained to North and South Conformations

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Carbocyclic sugars in modified nucleotides constrained to north/south conformations have A/B form (C3'/C2' endo) that can change the bending of RNA duplexes and rigidify nucleotides due to their locked sugar puckers. The dynamic behavior of an RNA dodecamer and an HIV kissing loop complex which contain several modified nucleotides with north/south carbocyclic sugars are being studied by explicit solvent molecular dynamics (MD) simulations. In the RNA dodecamer, two pairs of north carbocyclic sugars are substituted into the center region of dodecamer inducing an increased bending of the dodecamer axis. Similar bending behavior is also observed in an HIV kissing loop complex. Two pairs of north carbocyclic sugars are substituted into each stem of the HIV kissing loop complex and the angle between the kissing loop and both stems tips is reduced due to the north carbocyclic sugars. In order to rigidify the kissing loop complex, 12 north carbocyclic sugars are uniformly substituted into both stems and the average RMSD of the structure is found to be smaller than the unmodified kissing loop complex. In addition, it is observed that when a single strand of the RNA dodecamer contains north carbocyclic sugars, it can cause bending in the other unmodified strand. Stabilization of the kissing loop complex can also be obtained by substituting south carbocyclic sugar conformations into the flanking bulged-out base regions where it was found that the x-ray kissing loop complex had C2' endo south sugar conformations. These results suggest that proper use of north and south carbocyclic sugars can bend and stabilize RNA complexes without applying any external constraints.