places a cationic group in the major groove at the edge of a G-C pair. The low temperature NMR and x-ray crystal structures of some of these DNA appear identical to unmodified DNA; however, the thermodynamic analyses show that these modified bases have a significant impact on the dynamic structure of DNA. In most cases, a reduction in thermodynamic stability driven by enthalpy changes was observed. The only modification that is thermodynamically as, or more, stable than the corresponding unmodified DNA is the 7-aminomethyl-7-deaza-guanine. The thermodynamic effects of the different substitutions are associated with the folding enthalpies and hydration. Interpretation of how these base modifications affect DNA structure and stability will be discussed.

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220-Pos

Melting Behavior of DNA Complexes With Joined Triple and Duplex Motifs

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One focus of our laboratory is to understand how sequence, duplex and triplex stabilities, and solution conditions affect the melting behavior of complex DNA structures. We used a combination of UV and circular dichroism (CD) spectroscopies and differential scanning calorimetry (DSC) techniques to obtain a full thermodynamic description of the melting behavior of seven DNA complexes involving joined triplex and duplex motifs. Six of these complexes are formed intramolecularly while the seventh forms an intermolecular complex.

The circular dichroism spectra at low temperatures indicated that all complexes maintained the ''B'' conformation. UV and DSC melting curves of each complex show biphasic or triphasic transitions. However, the transition temperatures, T_Ms, of the intramolecular complexes remained constant with increasing strand concentration, while the T_M of the intermolecular complex did not. This confirms their molecularity.

Deconvolution of the DSC thermograms allowed us to determine standard thermodynamic profiles for the transitions of each complex. For each transition, the favorable folding free energy terms result from the characteristic compensation of a favorable enthalpy and unfavorable entropy contribution. The magnitude of these thermodynamic parameters (and associated T_Ms) indicate that the overall folding of each complex depends on several factors: a) the extent of the favorable heat contributions (formation of base pair and base triplet stacks that are compensated with both the ordering of the oligonucleotide and the putative uptake of protons and ions; b) inclusion of the more stable C^+GC base triplets; c) stabilizing the duplex stem of the complex; d) complex molecularity; and e) solution conditions, such as pHand salt concentration.

Overall, the melting behavior of each complex corresponds to the initial disruption of the triplex motif (removal of the third strand) followed by the partial or full unfolding of the duplex stem.

221-Pos

Formation and Quantification of Two-Photon Induced DNA Photolesions

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The generation of DNA photolesions with a high degree of spatial confinement presents unique opportunities to study the recruitment of UV damage repair proteins to localized damage sites. Photolesion formation is typically accomplished by exposure to UV light which is difficult to manipulate with conventional optics, thus limiting the spatial control over the site of irradiation. As an alternative, we use two-photon absorption of visible light to mimic UV exposure in a form that can be manipulated by conventional optics. We frequency double the output of a tunable Ti:sapphire laser using a barium borate crystal to generate femtosecond pulses of 340-540 nm light. Sample irradiation is performed on 10-20μL volumes confined in a multiwell plate and scanned by a focused beam in a raster pattern through different axial planes. We have adapted a sensitive PCR-based assay to quantify the amount of two-photon induced damage. The assay is premised on a reduction in DNA transcription efficiency by the presence of bulky photolesions; decreased amplification of a sample relative to a control indicates the amount of damage. The assay and laser irradiation system are being tested on linearized pBR322 plasmid, and validated by comparison to direct UV exposure. Our preliminary results indicate that the degree of lesion formation exhibits a nonlinear dependence on power, which is in keeping with the intensity dependence expected for two-photon absorption. Additionally, maximal two-photon DNA damage occurs at wavelength lower than twice the single photon absorption maximum. We are analyzing our results to obtain quantitative information about the yield of photolesions generated by two-photon absorption.

222-Pos

6MI Enhanced Fluorescence in a Specific DNA Pentamer Sequence

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The development of fluorescent nucleoside analogs, which hydrogen bond in the same fashion as their counterparts and minimally distort the structure of duplex DNA, has greatly improved the amount of information that can be obtained from both steady-state and time-resolved fluorescence experiments. Reduction in quantum yield observed when probes are incorporated into an oligomer or a duplex limits their potential application. 6-methylisoxanthopterin (6-MI) is a fluorescent guanosine analog which H-bonds with cytosine similar to guanosine. Investigating the photophysical properties of the nucleoside analog; we discovered a pentamer DNA sequence (ATFAA; where F 6-MI) that exhibits an enhancement of fluorescence upon formation of duplex DNA. The enhanced 6-MI fluorescence within a duplex broadens the potential applications by allowing binding and other experiments to occur at nanomolar concentrations. Within, the sequence context of ATFAA, time-resolved measurements reveal that the fluorescent populations shift from 0.4 to 7.2 ns upon formation of duplex and the relative quantum yield increases from 0.2 to 0.8. This implied the pentamer ATFAA fluorescence enhancement is due to 6MI adopting a single conformation that is either ''flipped out'' from the duplex or sterically constrained. To further investigate the enhancement of fluorescence upon duplex formation, we characterized oligonucleotides local and global structure. Temperature melt and iodide quenching experiments support a model in which enhancement of fluorescence is due to a solvent inaccessible geometry of 6MI remaining H-bonded to cytosine. An increase in solvent accessibility and reduction in the quantum yield were achieved through the introduction of a 3′ bulge or mismatch in the highly fluorescent duplex; suggesting limited dynamics of the 6MI is due to steric hiderance on the 3′ side. This information can now be used to generate other sequence contexts in which 6-MI will exhibit enhanced fluorescence upon duplex formation.

223-Pos

Alteration of Nucleic Acid Fluorescence by An Extenal Molecule and Its Practical Application in Enzymology

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The low fluorescence yield of nucleic acids makes it necessary either to attach extrinsic fluorophores, or add fluorescent intercalators in the case of dsDNA. We have found that the precence of 3-bromopropan-1-ol enhances the fluorescence yield of adenine, adenosine, 6-methylpurine and 7-methyladenine. In contrast, guanine, hypoxanthine, cytosine and poly-Adenosine did not exhibit this effect. This is due to an apparent shift in pKa of these molecules. In this work, we will focus our attention on adenine. Monitoring fluorescence from adenine as a function of 3-bromopropan-1-ol concentration, we constructed a Benesi-Hildebrandt plot that revealed the formation of a 1:1 complex with an equilibrium constant and Gibbs free energy of K 1.7E-5 and ΔGo 28.7 kJ/mol, respectively. We determined the fluorescence yield of adenine to increase about two orders of magnitude once the complex is formed. A second aspect of our work was to explore practical applications of this phenomenon. The observation that hypoxanthine was not similarly fluorescence enhanced allowed us to observe the kinetics deamination of adenine catalyzed by the enzyme adenosine deaminase (ADA). The reaction involves the exchange of an amino group for a hydroxyl group. The standard assay for ADA relies on the difference of absorption measurements. This standard assay is of limited sensitivity, since the absorption spectra of the substrate and product are overlapping, and the magnitude of their extinction coefficients are similar. The method we are developing relies on fluorescence spectroscopy, which proves to be more sensitive and exclusively detects adenine. Via this method we were able to study the kinetics of this reaction and determine the Michaelis constant and Vmax. The production of hypoxanthine was confirmed using HPLC separation techniques.

224-Pos

Cationic Sequence Dependence in Nucleic Acid Structures

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Nucleic acids require cationic shielding to overcome inherent self-repulsive electrostatics. The cations that take this role are collectively referred to as screening ions and exchange with those in the bulk solution. Here molecular dynamics simulations were performed for a large variety of helical stems to investigate the behavior of cations around nucleic acids. We show that cations have specific affinity with high residence times for polypurine stretches. Polypurine tracts are implicated in viral physiology, ribosomal entry points, and as aptamers for divalent cations. Also the examination of HIV-1 TAR RNA core

helix has shown that there is a sequence dependent cationic localization toward the purine-rich run within the TAR duplex. A region of high ion affinity agrees very well with the position of the X-ray determined divalent cations within a fragment from the HIV-1 TAR RNA. We show that a unique sequestration of ions within the core helix occurred independently of a nucleotide bulge and solely based on sequence of the helix. Our results suggest a high propensity toward purine dependent colocalization of one to two cations distinct from those performing phosphate backbone screening.

225-Pos

Computational Exploration of Thermodynamics and Kinetics of Mobile Ions Around RNA Duplex

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Atomically detailed distributions of ions around an A-form RNA are computed. Different mixtures of monovalent and divalent ions are considered explicitly. Studies of tightly bound and of diffusive (but bound) ions around 25 base pairs RNA are conducted in a explicit solvent. Replica exchange simulations provide detailed equilibrium distributions with moderate computing resources (20 nanoseconds of simulation using 64 replicas). Magnesium ion distributions show significant near-RNA binding while sodium ion distributions are more diffusive. Predicted binding sites of at the RNA surface are in accord with structures from crystallography. Electric field relaxation is investigated. The relaxation due to solution rearrangements relaxes in tens of picoseconds, while the contribution of RNA tumbling continues to a few nanoseconds. Negative mobile ions can be found near the RNA but must be assisted by proximate and mobile cations. At distances larger than 16Å from the RNA center, a continuum model of RNA charge density and solution becomes accurate. At shorter distances, the structure of RNA (and ions) has significant impact on the pair correlation functions.

226-Pos

Photochemistry of DNA Fragments Via Semiclassical Nonadiabatic Dynamics

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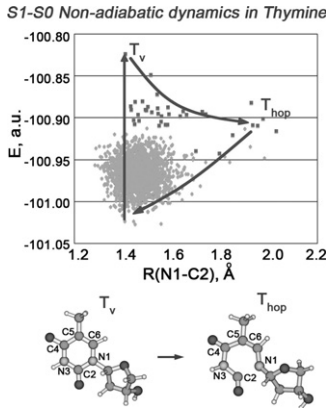
Forming upon absorption of a UV photon, excited states of DNA are subject to nonadiabatic evolution. Though photo-excited DNA mostly undergoes internal conversion back to the ground state, various routes of mutagenesis are also possible. Ultimately, the accumulation of errors in the genome can result in cancer. Here, nonadiabatic processes following the formation of the first singlet excited states, S1, in ten different small DNA fragments have been investigated: four single 4′H-nucleosides, two Watson-Crick base pairs, and four nucleotide quartets. Simulations were done via the nonadiabatic direct trajectory surface hopping semiclassical dynamics. The electronic wavefunction was obtained with configuration interaction, based on the semiempirical PM3 Hamiltonian with fractional orbital occupation numbers. The evolution of the electronic wavefunction was governed by the time-dependent Schrödinger's equation with a locally-diabatic representation, intrinsically stable near surface crossings. The nuclei evolved on adiabatic potential energy surfaces, as prescribed by classical Newtonian dynamics. The ''fewest switches'' surface hopping algorithm coupled the quantum and classical parts of the system. The dynamics simulations revealed several routes of nonadiabatic relaxation in these systems, which were not reported previously.



227-Pos

Application of Reptation Model on Brownian Dynamics for Electrophoresis of Single DNA in Polymer Solution

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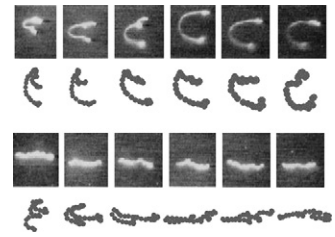
Brownian dynamcis(BD) simulation is performed to study electrophoretic motion of a single DNA moecule in polymer solution. When a DNA is forced to pass through pores in polymer solution under electrophoresis, the motion of DNA is strongly influence by surrounding entangled polymer molecules. We following the concept in the reptation model to represent the dynamics of DNA in polymer solution. Using the cubic Bezier spline, we manifest the contour of DNA to apply the constraint force from entangled polymer molecules surrounding the DNA. U-shaped, I-shaped migration, and periodic motions of DNA corresponding to each concentration of polymers solution under DC field, and the dynamics of DNA under AC field are simulated. We derive electrophoretic mobility using BD model with the constraint force to compare with experiment. We make the empirical correlation of the constraint force with concentration of polymer solution.



228-Pos

Partitioning of the Elastic Energy in Protein-Dna Chimeras

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We synthesize Protein-DNA chimeras where a DNA molecular spring mechanically perturbs the conformation of the protein. We measured the elastic energy stored in one such molecule, consisting of the enzyme Guanylate Kinase coupled to a 60 bp DNA spring. From these measurements, the response of the protein in terms of its enzymatic activity, and a mechanical model of the DNA spring we deduce that, in this case, most of the elastic energy of the molecule is stored in the DNA spring. Thus the DNA spring is ''softer'' than the protein.

229-Pos

Self-Assembly in a Model Amphiphile System

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The physical origin of the large and negative excess entropy of mixing of alcohols and water remains controversial. In contrast to standard explanations that evoke concepts of water structuring, recent work has shown that, at ambient conditions, it can be quantitatively explained in terms of molecular scale partial demixing of the two components. Here, we estimate the negative excess entropy of aqueous methanol at low temperature and high pressure using experimentally-derived structural data and a recently introduced cluster model. On cooling to 190 K the cluster sizes increase, but the change in negative excess entropy, which according to this method of calculation depends on the surface area to volume ratio of the clusters, is not significant, suggesting that the topology of the clusters must change with decreased temperature. On compression the cluster sizes also increase, and the negative excess entropy is now positive, suggesting an even more pronounced change in cluster topology with increased pressure. This work suggests that it is the amphiphilic nature of a molecule that determines aggregation and self-assembly processes in aqueous solution.
The results therefore give useful insight into the processes of cold and pressure denaturation of proteins.

230-Pos

Hydrophobic and Hydrophilic Interactions

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We are trying to understand mysteries of nature by looking at extra large (Cosmology and Astrophysics) and extra small distances (Weak/Strong interactions High energy Physics) while it can be seen in the physics of life systems. In previous presentations I developed some theoretical vision about conformational motion and non-equilibrium dancing of biological macromolecules by giving definitions of non-equilibrium entropy and geometrical motion. Those two key definitions and formulations are believed to be enough to answer the questions what is big energetic fluctuation is induced by in non-equilibrium systems and how the life system functions properly under that fluctuations. Self-organization is dynamic process where hydrophobic-hydrophilic interactions take a crucial role. Non-equilibrium dancing may induce 'hydrophobic-hydrophilic' waves which may be felt by other molecules. One may think that the suggestion about conformational motion may complicate quantitative and qualitative description of hydrophobic-hydrophilic interactions. Nevertheless, geometrical motion itself indicates changes of hydrophobicisity of the surfaces and can be completely described if it is taken into account dynamic processes of the surfaces solvent interactions. Since, in solvents, we have large number of interacting molecules statistical physics supposed to have crucial role in describing those dynamic processes but tusk is complicated by non-equilibrium nature of the processes. Fluctuation theorem guarantees reversibility of non-equilibrium processes but caries probabilistic nature so can not strictly predict whether entropy will decrees or increase in time. The problem becomes solvable in