however, the processes underlying this important feature of meiosis are not yet understood. In this work, we show that even in the absence of proteins or additional polymers, individual dsDNA molecules of 5 kb or longer in solutions containing only monovalent ions, can self-assemble into sequence dependent pairs, where this homologous dependent pairing can occur at lower dsDNA concentrations than those typically found in vivo. We have used magnetic tweezers to probe the stability of these molecular pairs and demonstrate that at room temperature they remain bound for shear forces up to 10 pN. To elucidate the mechanisms that underlie the observed homologous association of pairs, we studied the effect of sequence, dsDNA concentration, non specific competitors, temperature, and salt.

1773-Pos Board B617

Conformational Equilibria Of Bulged Sites In Duplex Dna Studied By EPR Spectroscopy And Differential Scanning Calorimetry

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Conformational flexibility in nucleic acids provides a basis for complex structures, binding, and signaling. One-base bulges directly neighboring single-base mismatches in nucleic acids can be present in a minimum of two distinct conformations, complicating the examination of the thermodynamics by calorimetry or UV-monitored melting techniques. To provide additional information about such structures, we demonstrate how electron paramagnetic resonance (EPR) active spin-labeled base analogues, base-specifically incorporated into the DNA, are monitors of the superposition of different bulge-mismatch conformations. EPR spectra provide information in terms of ''dynamic signatures'' that have an underlying basis in structural variations. By examining the changes in the equilibrium of the different states across a range of temperatures, the enthalpy and entropy of the interconversion among possible conformations can be determined. The DNA constructs with a single bulge neighboring a single-base mismatch (''bulge-mismatches'') may be approximately modeled as an equilibrium between two possible conformations. Experiments on the bulge-mismatches show that basepairing across the helix can be understood in terms of purine and pyrimidine interactions, rather than specific bases. Measurements of the enthalpy and entropy of formation for the bulge-mismatches by differential scanning calorimetry and UV-monitored melting confirm that the formation of bulge-mismatches is in fact more complicated than a simple two-state process, consistent with the base-specific spectral data that bulge-mismatches exist in multiple conformations in the pre-melting temperature region. We find that the predictions of the nearest-neighbor (NN) model, based on data from DNA denaturation experiments, do not correlate well with the structures inferred from the base-specific EPR dynamics probe. We report that the base-specific spin probes are able to identify a bi-stable, temperature dependent, switching between conformations for a particular complex bulged construct.

1774-Pos Board B618

Closing The Lid On Dna End-to-end Stacking Interactions

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Recent experiments suggest that short DNA strands associate by end-to-end stacking. The stacking of hydrophobic ends of double-strand DNA molecules may provide the favorable free energy required for association. Here, we report interactions between DNAs with modified ends. DNA duplexes, 20 bp long, were capped with short T_4 loops at 2, 1 or 0 ends, and were placed in solutions dialyzed against buffer containing 20mM Mg^{2+}. Association was not observed in constructs with both ends capped. DNA-DNA interactions were characterized by measuring variations in small angle X-ray scattering (SAXS) curves at the lowest scattering angles. Second virial coefficients were computed from the SAXS data to further confirm that end-to-end stacking plays an important role in short strand DNA-DNA interactions.

1775-Pos Board B619

A Designed 3D Self-Assembled Crystalline DNA Array

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The precise control of the structure of matter is a central concern of the natural sciences. To this end, numerous investigators have developed self-assembling systems to produce targets of interest. Taking its cue from biological systems, structural DNA nanotechnology has used branched DNA motifs combined with cohesive ends to produce objects, nanomechanical devices and designed two-dimensional lattices. The details of these 2D lattices have been studied primarily by atomic force microscopy, whose resolution is 3-10 nm in typical analyses. The criteria for three dimensional self-assemblies are more strict, because the primary technique for their analysis is x-ray crystallography, whose resolution is limited only by the wavelength of the source (about 1 A). Previous efforts to produce self-assembled three-dimensional lattices have produced lattices that conformed to the design, but whose resolution was no better than 10 A, not really capable of revealing molecular structure. Here, we report the crystal structure at 5 A resolution of a self-assembled designed three-dimensional lattice based on the tensegrity triangle. Each edge of the tensegrity triangle contains two turns of DNA. The structure and sticky ends have been designed to be 3-fold symmetric, and at this resolution it appears to be so. The data are of sufficient quality to demonstrate clearly that it is possible to design a 3D lattice using the techniques of self-assembly based on molecular recognition.

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1776-Pos Board B620

Helix-Coil Transitions of Unusual DNA Structures by Measuring the Fluorescence Changes of 2-Aminopurine When Incorporated into DNA

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One focus of our research is to investigate the helix-coil transition of unusual DNA structures by comparing optical and calorimetric melting curves with fluorescence melts obtained by observing the fluorescence changes of 2-aminopurine (*AP*) when incorporated into DNA. In this work, we used a combination of temperature-dependent UV, circular dichroism (CD) and fluorescence spectroscopies, and differential scanning calorimetric (DSC) techniques to investigate the unfolding of: a) an intramolecular pyrimidine triplex, $A_3APA_3C_5T_7C_5T_7$; b) a G-quadruplex, $G_2T_2G_2TAPTG_2T_2G_2$; and c) a dodecamer duplex, CGCGA*AP*TTCGCG. Our experimental observations are as follows.

a) All melting curves of the triplex show similar monophasic transitions with T_Ms of 31 °C, ΔH_{vH}s of 38 kcal/mol and ΔH_{DSC} of 70 kcal/mol. Thus, this triplex unfolds in a non-two state fashion similar to the unfolding of the unmodified triplex but with a lower endothermic heat (by 12 kcal/mol). This indicates the presence of *AP* contributes with lower stacking interactions.

b) The G-quadruplex also unfolds in similar monophasic transitions: T_Ms of 52°C, ΔH_{vH}s of 44 kcal/mol and ΔH_{DSC} of 36 kcal/mol. The unstacking of *AP* follows the overall cooperative unfolding of the whole molecule. This quadruplex unfolds in two-state fashion similar to the unfolding of the unmodified quadruplex but with a higher endothermic heat (by 14 kcal/mol). This indicates that the presence of *AP* induces higher stacking contributions.

c) All melting curves of the dodecamer duplex show biphasic transitions. The T_M of the first transition depends on strand concentration while the T_M of the second one is independent, consistent with the presence of a duplex ← → hairpin ← → random coil equilibria. The central placement of *AP* destabilizes the duplex state, while does not affect the hairpin state.

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1777-Pos Board B621

Understanding the Chemistry of Cytosine Methylation Effects

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Proteins bind to DNA to initiate several important biological processes including DNA replication and transcription of genes, these processes are also highly regulated. A common method of bioregulation is cytosine methylation. Recent research suggests that cytosine methylation quenches DNA dynamics, preventing the DNA deformation often necessary for efficient protein-DNA complex formation. Using ^{31}P NMR, we have shown that methylation induced rigidity may stem from conformational changes in the backbone from BI to BII conformation. To explore the mechanism of these conformational changes, we have turned to the study of cytosine fluorination.

Through comparison of FTIR and NMR spectra, respectively, one can qualitatively and quantitatively observe the effects of methylation and fluorination on DNA structure and dynamics. Preliminary results demonstrate that these different covalent modifications of the cytosine base affect the structure and dynamics in different ways. Both modifications alter the BI/BII ratio but fluorination does not appear to affect the dynamics as significantly as methylation. We attribute the enhanced quenching of backbone dynamics by methylation to the formation of a strong dipolar interaction between the negatively charged backbone oxygen with the hydrogens of the methyl group whose proximity to the backbone is closer in BII than BI. Comparisons of fluorination and methylation effects on sequences containing both the EcoRI and Cre binding sites, as well as their modulation due to sequence context, will be presented.

1778-Pos Board B622
Enthalpy-Entropy Contribution to Carcinogen-induced DNA Conformational Heterogeneity
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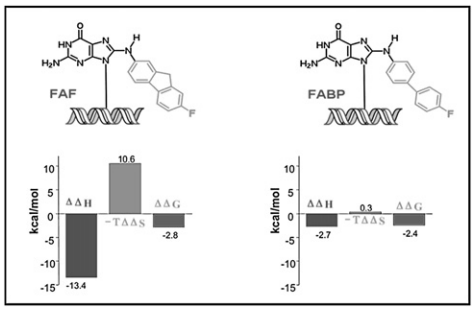
Aromatic amines are among the most notorious chemicals in the environment. The formation of DNA adducts is thought to be a hallmark for the initiation of chemical carcinogenesis. Aromatic amine-DNA adducts are known to exist in a sequence-dependent equilibrium of the major groove B-type (B) and base-displaced stacked (S) conformations. We have conducted extensive calorimetry/NMR studies on the model lesions FAF and FABP in order to understand how thermodynamics influence the nature of S/B-conformational heterogeneity and subsequent molecular interactions with polymerases and repair proteins. Results indicated large differences in enthalpy-entropy compensations for FABP and FAF. The small and flexible FABP exclusively adopts the less perturbed B-conformer, thus resulting in small enthalpy/entropy change. This is in contrast to FAF, which stacks better and exists as a mixture of B- and S-conformers, thus contributing to large enthalpy/entropy compensation. The results indicate that it is not just the stacking argument, but also the favorable entropy of the S-conformer over B-conformer that determines the S/B-conformational heterogeneity at an ambient temperature.



1779-Pos Board B623
Scaling Behavior of Single Stranded DNA Measured by Small Angle X-ray Scattering
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Polyelectrolytes, or charged polymers, are prevalent in biological systems, yet their physical properties are far less well understood than those of neutral polymers. We report on measurements using small angle x-ray scattering to study bulk ensemble-averaged properties of small (up to 100 bases) poly-deoxythymine (poly-dT) and poly-deoxyadenine (poly-dA) molecules. By studying homomeric single stranded DNA (ssDNA), we can observe their polymeric properties without interference from secondary structure formation. This gives us insight to the conformational space explored by single stranded nucleic acids in folding processes, and the nucleotide dependence of loop flexibility of DNA and RNA junctions. We observe, as is consistent with base-stacking of purines, that poly-dA is stiffer than poly-dT. For poly-dT, the radius of gyration (R_g) scales with the number of monomers with a Flory exponent (ν) which decreases slowly with increasing salt, but drops sharply below that expected for a self-avoiding random walk (SAW) polymer ($\nu \sim 0.588$) with more than 500 mM of added sodium acetate. This is perhaps due to the condensation of charges around the DNA and/or the change in solvent quality with added salt. The ratio (r) of the square of the maximum pair-wise distance (D_{max}) to R_g fluctuates around 10, suggesting that ssDNA compacts locally. Assuming that D_{max} is a fair estimate of the end-to-end distance of the polymer, a value of 12 is expected for r in the case of a rod, and about 6.3 for a SAW polymer. This localized clustering is consistent with the electrostatic blob model of de Gennes *et al.* The persistence length of poly-dT - determined by fitting the data to a worm-like chain model - increases in a sublinear fashion with increasing Debye screening length, unlike the behaviors predicted by polyelectrolyte theories.

1780-Pos Board B624
Macrosolute Effects on Nucleic Acid Interactions
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The intracellular environment contains a variety of solutes that cumulatively occupy a significant volume of the cell (20-30%). The high volume occupancy generates a system which is macromolecularly crowded. This crowding, also known as the excluded volume effect, can lead to an increase in the chemical activity of solutes and influence thermodynamic and kinetic values as compared to a dilute system. Using synthetic, inert cosolutes to provide a simplified mimic of the crowding in the intracellular environment, DNA structures were studied. We demonstrate that crowding can lead to the differential stabilization of a complementary DNA duplex over duplexes containing a single mismatched base pair, effectively increasing the specificity of the hybridization reaction. In systems with molecularities ranging from one to four we demonstrate that as the molecularity of a system increases the crowding effects also increase. An increase in Tm of up to 12°C was noted for a multi-branch DNA structure with four arms. Crowding mediated enhancement of the rate of hybridization was found to be independent of sequence but dependent upon structure.

1781-Pos Board B625
The Interaction of Monovalent Cations with a Model DNA Hairpin
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Capillary electrophoresis was used to study the interactions of monovalent cations with DNA hairpins, using as a model the 16 residue oligonucleotide ATCCTATTTTAGGAT, which is known to form a stable hairpin with a 6 base pair stem and a 4 base loop. The unstructured 14 base oligonucleotide ACCTGATCACGTTA served as a reference analyte. All measurements were performed in the absence of Mg^{2+} at pH 7.3 using diethylmalonate as the buffering anion. Increasing the concentration of Na^+ in the buffer increased the melting temperature of the hairpin, as predicted by the mFOLD algorithm. Isothermal measurements at 20° indicate that Na^+ forms a saturable complex with the hairpin, with a K_D of about 100 mM, but does not form a complex with the unstructured reference oligonucleotide. These measurements suggest that the increase in the melting temperature of the hairpin with increasing Na^+ is due to the preferential binding of Na^+ ions to the hairpin conformation. The cations Li^+, K^+, $Tris^+$ and tetramethylammonium$^+$ (TMA^+) bind equally well to the model hairpin and affect its melting temperature similarly. The tetraethylammonium$^+$ (TEA^+) ion also binds equally well to the hairpin, but only to the extent of ~50% saturation. The tetrapropylammonium$^+$ (TPA^+) and tetrabultylammonium$^+$ (TBA^+) ions bind to the hairpin very weakly if at all. Surprisingly, the melting temperature of the hairpin is systematically diminished as TMA^+ is replaced in turn by TEA^+, TPA^+ or TBA^+, suggesting that the larger tetraalkylammonium ions may destabilize the hairpin conformation by a through-solvent mechanism.

1782-Pos Board B626
Observation of Oligonucleotide Dynamics by means of Fluorescent Nucleoside analog 6MI
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To improve current understanding of the structural recognition mechanism of architectural DNA binding proteins such as HU and IHF, we are investigating the structure and dynamics of different DNA substrates. We are able to make these observations on both global and local levels by incorporating the fluorescent guanosine nucleoside analog 6-methylisoxanthopterin (6-MI), with H-bonds with cytosine similar to guanosine. We have previously shown this probe does not significantly perturb the global structures of duplex DNA molecules. 6-MI was systematically incorporated into a 34 base oligonucleotide. Initial characterization of local DNA environment included time resolved fluorescence and rotational correlation measurements of the duplex oligomers relative to 6-MI monomer and single stranded DNA. Analysis of time-resolved fluorescence decay yields 3 lifetime components of 0.4 ns, 4 ns and 6.5 ns. The largest