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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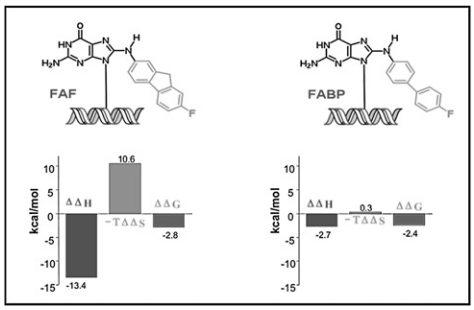
Aromatic amines are among the most notorious chemicals in the environment. The formation of DNA adducts is thought to be a hallmark for the initiation of chemical carcinogenesis. Aromatic amine-DNA adducts are known to exist in a sequence-dependent equilibrium of the major groove B-type (B) and base-displaced 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

lived component is similar to that of 6-MI monomer, 7 ns. The position of the probe shifts the fluorescent populations from 0.4 ns to 6.5ns upon formation of duplex, which implies that 6-MI local environment in these positions resembles that of the solvent exposed monomer. However, no direct correlation between adjacent base sequence and the fluorescent properties of 6MI was observed. To further investigate the increase in fluorescence upon duplex formation, we characterized the local and global structure of several oligonucleotides through temperature melts, quantum yield calculations, quenching assays, and Raman spectroscopy. The results suggest that, the position of 6-MI in the duplex sequence, helical turn, and surrounding base sequence determines the dynamics of 6-MI. This potentially leads to the formation of a fixed geometry of 6-MI which stacks poorly with adjacent bases. The lack of stacking interactions causes 6-MI to exhibit fluorescent properties of the monomer.

1783-Pos Board B627

Single Base Interrogation by a Fluorescent Nucleotide: Each of the DNA Bases Identified by Fluorescence Spectroscopy

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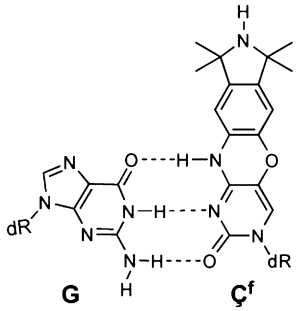
The fluorescent nucleoside (fluoroside) $\mathbf{Ç^f}$ is a cytosine-analogue that forms a stable base-pair with deoxyguanosine in DNA.

The fluoroside is able to report the identity of its base-pairing partner in duplex DNA: A different fluorescent signal is obtained when paired with A, G, T or C (Cekan P and Sigurdsson ST (2008), *Chem. Comm.*, *29*, 3393-3395). In addition, $\mathbf{Ç^f}$ shows appreciable fluorecence, even when flanked by a G/C pair, which has been reported to substantially diminish fluorescence of other fluorosides. Stern-Volmer titration with the four nucleoside triphosphates indicates that the discrimination originates from direct interaction of $\mathbf{Ç^f}$ with its base-pairing partner, rather than from different exposure of $\mathbf{Ç^f}$ to the solvent. These properties make $\mathbf{Ç^f}$ a promising candidate for the detection of single nucleotide polymorphisms (SNPs). To evaluate the possible use of $\mathbf{Ç^f}$ for SNP typing, we have systematically deterimined the effects of flanking sequence on mismatch detection. We have also studied the effects of other experimental variables, such as solvent polarity, on fluorescence.



1784-Pos Board B628

Identification of Phase Transition and Twist-Stretch Coupling During DNA Supercoiling

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As a single DNA molecule is supercoiled under tension, the onset of a phase transition is most definitively revealed by a torque plateau. However, in the absence of direct torque detection, a change in the observed extension has often been used to identify the onset of a phase transition. Here by directly measuring torque using an angular optical trap, we show that an extension maximum, which has previously been assumed to be indicative of the onset of a phase transition from a B to sc-P-form DNA, in fact does not coincide with the onset of a torque plateau at a phase transition. Instead this maximum is well explained by a theory by John Marko that incorporates both DNA twist-stretch coupling and bending fluctuations. This theory also provides a more accurate method to determine the value of the twist-stretch coupling modulus, which is underestimated without consideration of the bending fluctuations as was done in previous studies. Our study demonstrates the importance of torque detection in the identification of phase transitions as well as the contribution of the bending fluctuations to DNA extension.

1785-Pos Board B629

Using Polymer Models To Understand The Structure Of Chromosome III In Budding Yeast

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Eukaryotic and prokaryotic cells have intricately structured chromosomes. Their large-scale physical structure is believed to arise from protein-mediated interactions that can form both inter- and intra-chromosomal tethers as well as anchoring the chromosome to the membrane or purported protein scaffolds. While classical molecular biological techniques have yielded information about the structure and chemistry of chromosomes, little is known about their conformations or dynamics *in vivo.* To study this, we have placed fluorescent markers in *Saccharomyces cerevisiae* near the HML locus on chromosome III and at the spindle pole body, which serves as a marker of centromere attachment, and measured the cell-to-cell distribution and dynamics of distances between these two loci. The histograms of distances obtained in this way were analyzed using a model of the chromosome as a random-walk polymer. To account for the measured distance distributions, we conclude that the motion of the left arm of chromosome III is constrained by the presence of a tether. This motivates experiments in which known tethering mechanisms for chromosome III are removed and the effect on the distance distributions is measured. We report on such experiments using strains in which the HML locus was deleted, ones in which a known telomere tethering protein was removed, and strains with a deletion of 17kb of DNA between HML and the centromere.

Membrane Physical Chemistry II

1786-Pos Board B630

The Effect of Shear Stress on the Fluidity of Supported Lipid Bilayers

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Previous studies show that fluid shear stress on cell membranes causes metabolic changes in cells. One question that arises is what physical effects shear stress has on the fluidity of artificial lipid bilayers. As demonstrated by Haidekker *et al*, shear stress can change membrane fluidity of endothelial cells within 5 seconds, and membrane fluidity recovered completely after flow stopped. Due to the speed and reversibility of the change in membrane fluidity caused by shear stress, we hypothesized that the change in fluidity may be caused by the direct effect of shear stress on the packing of the lipid bilayer itself, rather than an effect mediated through an intracellular signaling cascade. This research studied the effect of shear stress on a protein-free membrane by inducing shear stress on a supported lipid bilayer in a micro-volume flow chamber. The effect of changing fluid flow in step increments on the membrane fluidity was measured using z-scan fluorescence correlation spectroscopy (FCS).

1787-Pos Board B631

Ordered Suspensions of Charged Liposomes

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Accurate determination of liposome and colloidal-vesicle charge remains an unsolved problem. Electrophoretic measurements are subject to slip-plane and surface-structure uncertainties. We propose a charge measurement that avoids hydrodynamics in the vicinity of the shear layer so is independent of the slip plane, eliminates diffuse-layer electroosmosis by avoiding applied electric fields, and is insensitive to surface structure. We form ordered electrostatic gels of monodisperse unilamellar PG/PC liposomes by high-pressure extrusion through polycarbonate membranes in deionized water. The gel consists of a strongly-coupled suspension of liposomes, stabilized by mutual long-range electrostatic repulsions in a confined volume. The gel structure is that of an ordered liquid, as seen by angle-dependent static light scattering, freeze-fracture electron microscopy, and optical Bragg scattering. The liposome charge Z is screened by dissociated H^+ counterions. Z thus determines both the strength and range of the interactions, hence determines the elastic properties of the gel. Measurements of shear modulus by a mechanical resonance technique and of osmotic compressibility by dynamic light scattering yield an effective particle charge Z*. The relation of Z* to Z may be calculated by numerical iteration of the Poisson-Boltzmann equation for any Z* and particle concentration. The technique is most accurate in the Debye-Hückel regime where $Z \sim Z^*$. Addition of H^+ and salt permits measurement of protonation and counterion binding to the surface. This method of determining liposome charge by measuring the bulk elastic properties of a strongly-coupled suspension differs markedly from that of measuring the mobilities of individual liposomes in an uncoupled suspension.