

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 6.5 ns 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.

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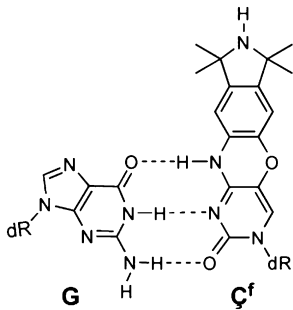
Single Base Interrogation by a Fluorescent Nucleotide: Each of the DNA Bases Identified by Fluorescence Spectroscopy

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The fluorescent nucleoside (fluoroside) ζ^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, ζ^f shows appreciable fluorescence, 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 ζ^f with its base-pairing partner, rather than from different exposure of ζ^f to the solvent. These properties make ζ^f a promising candidate for the detection of single nucleotide polymorphisms (SNPs). To evaluate the possible use of ζ^f for SNP typing, we have systematically determined the effects of flanking sequence on mismatch detection. We have also studied the effects of other experimental variables, such as solvent polarity, on fluorescence.



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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).

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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.