3419-Pos
Spectroscopic Studies of DNA-Drug Binding Affinities: Sequence Context and Methylation Effects
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Fluorescence spectroscopy studies of 7-Aminoactinomycin D (7-AMD) binding to dodecamers containing the *Cre* binding sequence demonstrate that binding affinity is directly related to both sequence context and cytosine methylation. Importantly, it was also found that methylation effects on binding affinity are sequence dependent. The native d[TTTCACGTGAAA]$_2$ and d[AAGAACGTTCTT]$_2$ samples show very similar binding affinities in their native forms, while native d[GAAAACGTTTTC]$_2$ has a dissociation constant four-fold higher than either of these samples. Upon methylation, d[TTTCA-m^5-CGTGAAA]$_2$ shows the greatest change in the dissociation constant, increasing two-fold. The d[AAGAA-m^5-CGTTCTT]$_2$ sequence has a 1.5-fold increase in the dissociation constant, while the d[GAAAACGTTTTC]$_2$ sequence shows no significant change in binding affinity upon methylation. NMR and FTIR evidence will be presented to further explain these phenomena and provide evidence that backbone conformations due to flanking sequence and methylation state are responsible for these differences.

3420-Pos
Solution Structures and Ultraviolet Raman Spectra of Purines Reveal Systematic Shifts with Change in Protonation State
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Purines and pyrimidines that form the nucleobases in normal and modified DNA and RNA are known to exist in several tautomeric and ionization states. The relative populations of tautomers in solution and their precise structure are highly sensitive to ring substitution and pH. Knowing the structures in different environments is essential to understand the detailed chemistry of purine modifying enzymes such as ribosyl transferases and DNA glycosylases. We used ultraviolet Raman spectroscopy in resonance with nucleobases and exhaustive DFT calculations to reliably identify solution structures of several nucleic acid enzyme substrates at different pH and established their vibrational signatures. We find that the DNA damage lesion 8oxoguanosine is in the diketone form at physiological pH. At high pH, the anion is formed via deprotonation at N1 while the enolic form was not detected. Hypoxanthine exists as a mixture of the N7H and N9H forms though xanthine exists as a single diketone tautomer in solution. Xanthine is neutral at pH 7 but pKa changes accompany formation of the corresponding mononucleotide which is found to exist predominantly in the deprotonated form at this pH. The vibrational spectra show systematic shifts correlated with protonation state with an overall reduction in the wavenumbers of the purine ring vibrations upon deprotonation. The spectral signatures of purines established here can be used to selectively observe nucleic acid-protein interaction.

3421-Pos
Preferential Site Binding of Monovalent Cations With the Random Coil Form of DNA
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Collins and Rogers (Chem. Biol. Interactions *19* 197 1977) have suggested that monovalent tetraalkylammonium (TXA^+) ions diminish the melting temperature of duplex DNA by preferential binding to the random coil form. We have investigated this suggestion using a known 16 base hairpin as the test oligomer and electrophoretic mobility in free solution as the analytical parameter. Measurements were made in the range 15-60° using background electrolyte solutions (BGE) containing 0.01-0.9 M monovalent cation (M^+) and diethylmalonate as the buffering cation at pH 7.3. A 16 base all-T oligonucleotide served as a reference random coil to correct for changes in hairpin mobility contributed by changes in the physical properties of the BGE. Thermal transitions were observed as a decrease in the mobility ratio of the hairpin/coil to 1.00 as the temperature was increased at a fixed [M^+]. The Tm of the hairpin increased hyperbolically from 37° as the [M^+] increased linearly. The increase in Tm observed with Na^+ is exactly that predicted by the DINAMelt algorithm. The span of the increase in Tm has the order Na=Li=K>NH4>TMA>Tris>TEA>TPA>TBA. Cation site binding was analyzed as a change in the mobility ratio with increasing [M^+] at 20° where the hairpin conformation predominates. The mobility ratio increases hyperbolically with increasing [M^+], consistent with the view that M^+ preferentially site binds with the coil form. The magnitude of the increase in mobility ratio from the binding measurements at 20° correlates with the dTm/d(log M^+) from the melting measurements, suggesting that the two phenomena are related. We propose that the effect of M^+ on DNA melting is the net result of two opposing contributions, preferential stabilization of the counterion cloud about the structured form and preferential site binding with the unstructured random coil form.

3422-Pos
Measuring DNA Condensation Driven by Cobalt Hexamine
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We investigate the mechanism of DNA condensation mediated by trivalent cobalt hexammine (cohex) in solution, using 3 established experimental techniques. The amount of DNA precipitated out of the solution can be calculated by directly measuring the change of the UV absorption of the supernatant. Inter-DNA attraction is monitored by changes in the low angle region of small angle X-ray scattering (SAXS) profiles. Wide angle X-ray scattering (WAXS) is also applied to explore shorter length scales. Comparison with simulations reveals details of cohex association to DNA. Our results suggest that inter DNA attraction depends on the length of the helix and the association mode of the ions.

3423-Pos
A Reversible Switch on Two-Dimensional Small Interfering RNA Condensation
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Small interfering RNAs (siRNAs) are short (19-29bp) double stranded nucleic acids that efficiently mediate gene silencing in mammalian cells by directing the degradation of complementary target mRNA sequences. This has justified the recent development of technologies for siRNA transport into a host cell. Synthetic cationic lipid (CL) assemblies can efficiently be used to deliver siRNA, leading to highly specific gene silencing [1].
The ability of CL-siRNA constructs to efficiently knockdown genes is strongly correlated with the packing of the nucleic acid molecules within the lipid bilayer. We used Synchrotron X-ray diffraction to show that CL-siRNA self-assembly may lead to the formation of distinct 2D phases. This includes condensed 2D smetic and isotropic phases with reversible transitions between them mediated by a combination of electrostatic and thermal fluctuations effects.
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[1] Bouxsein et al. Biochemistry (2007) 46, 4785.

3424-Pos
New Degradable Cationic Lipid-DNA Complexes for Gene Delivery
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Cationic lipids (CLs) continue to attract attention as synthetic nucleic acid (NA) vectors, and are broadly used for gene transfection and silencing including applications in clinical trials. However, these complexes exhibit suboptimal gene expression due to inefficiencies in overcoming the cellular barriers to transfection (Ahmad et al., J. Gene Med. 2005, 7, 739). After entry of the CL-DNA complex into the cell, two major barriers are efficient dissociation of DNA from the complex and the cytotoxicity of CLs. To address both these issues, we have synthesized a novel series of multi¬valent lipids (CMVLs) with degradable disulfide bonds linking the positively charged headgroups of the CMVLs to their hydrophobic tails. The linker is designed to be cleaved in the reducing milieu of the cytoplasm thus facilitating CL-NA complex degradation, reducing cytotoxicity and improving NA release. X-ray scattering demonstrates that CMVLs form lamellar complexes with DNA, which degrade in reducing environments mimicking the cytoplasmic milieu. For lipids with highly charged headgroups such as CMVL5 (5+), X-ray diffraction under reducing conditions shows DNA condensed by the cleaved headgroup. No such condensation is observed for smaller headgroup charge, as in CMVL2 (2+). Most significantly, we observed an unexpectedly large reduction in cytotoxicity of degradable CMVL-vectors compared to vectors prepared from corresponding lipids without degradable bonds (MVLs). This is of particular importance for applications in gene silencing, where the delivery of short interfering RNAs (siRNAs) requires large CL/NA charge ratios. Correspondingly, the transfection efficiency of CMVL-DNA complexes remains high for high CL/DNA charge ratios. In summary, the much reduced cytotoxicity of these new degradable multivalent lipids and their propensity for DNA release in the cytoplasm open the way for the development of efficient non toxic CL-vectors of NAs. Funding provided by NIH GM-59288.