2961-Pos Board B8
Mechanism Of Monovalent Counterion Specificity In A RNA Kissing Loop Complex
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RNA tertiary structure is often stabilized by ''kissing hairpin'' motifs. We report findings from molecular simulations performed on Tar-Tar*, an archetypal kissing hairpin complex composed of two complementary 16 nucleotide stem-loops. The kissing loop interface has an unusually high density of backbone phosphates, which is thought to form a ''cation-binding pocket''. Draper and coworkers have shown that stability of the Tar-Tar* complex is highly sensitive to the identity of the cation amongst the series of alkali-chloride salts, (CsCl < KCl < NaCl).
We have calculated preferential interaction coefficients the free energies of ion binding to various regions of the complex using spatial density functions (SDFs). The main results are as follows: The central core is the most favorable region for counterion binding. However, irrespective of counterion type, the occupancy counterions in the high density pocket is always 2 implying that site-specific binding alone does not explain observed specificities. The SDFs reveal that both the complex and the isolated hairpins exhibit a curious asymmetry, i.e., there is greater accumulation of cations around Tar as opposed to Tar*. This turns out to be a consequence of an asymmetry at the sequence level, as the Tar hairpin contains a guanidine tract, which efficiently replaces lost first shell waters of partially dehydrated ions that accumulate at the core. The results suggest that experimentally observed counterion specificities of Tar-Tar* stability originate in preferential exclusion of the Cl- coion around the complex, which is most pronounced in the NaCl as opposed to KCl and CsCl. Coion depletion also facilitates counterion delocalization to form a stabilizing belt around the complex thereby alleviating the inherent asymmetry in a priori counterion accumulation; again Na+ achieves this delocalization most efficiently and is followed by K+ and Cs+, respectively.

2962-Pos Board B9
TMAO and Solvent Exposed RNA Bases Stabilizes Unfolded State via Hydrogen Bonding
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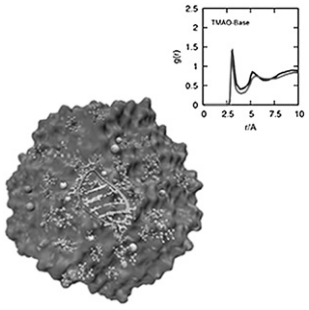
TMAO is an osmolyte that accumulates in cells in response to osmotic stress, and its role in the stabilization of proteins has been well characterized but its effect on RNA is less understood. In our study, we investigate how TMAO interacts with a 12-nt RNA hairpin with an exceptionally high melting temperature and an 8-nt RNA hairpin with a relatively fluid native basin in the absence of TMAO. The two hairpins correspond to folded and unfolded states, respectively, at physiological temperatures. We performed multiple explicit water all-atom molecular dynamics (MD) simulations of the RNA hairpins using AMBER and CHARMM force fields, both in the absence and presence of TMAO. We observed remarkably similar RNA-TMAO interactions from the simulations using the different force fields. The radial distribution functions between the oxygen hydrogen bond acceptor in TMAO and every possible hydrogen bond donor in the RNA hairpins show that TMAO preferentially interacts with only hydrogen bond donors that are in the base, only if they are solvent exposed like when the RNA is not in the folded state. Our simulations help explain recent experiments that show TMAO stabilizes RNA.



2963-Pos Board B10
Single-Molecule FRET Studies of Lysine Riboswitch Folding
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Riboswitches, found in the 5′ untranslated regions of bacterial messenger RNA (mRNA), regulate gene expression through secondary or tertiary structural changes induced by metabolite binding. Localized conformational changes near the metabolite-binding site alter downstream secondary structure, ultimately determining gene expression. This ribo-regulation of gene expression depends significantly on the kinetics and thermodynamics of the structural changes of the mRNA and metabolite binding, and therefore a thorough understanding of these dynamics is crucial to developing a complete knowledge of the riboswitch functionality. In an effort to probe the folding interactions of riboswitches, the 208-nucleotide metabolite-binding domain of the Bacillus subtilis lysine riboswitch was prepared by transcription and hybridization to a dual fluorescent label RNA strand, which allowed FRET monitoring of structural changes. Ensemble measurements revealed structural sensitivity to monovalent (Na+, K+) and divalent (Mg2+) cations. The RNA construct was also studied using time-resolved-single-molecule FRET (smFRET) methods that allow for thorough characterization of the riboswitch-folding dynamics under equilibrium conditions. The effects of cations on the tertiary folding/unfolding of the lysine riboswitch were investigated. Single-molecule studies were performed on freely diffusing riboswitches, as well as, riboswitches tethered to surfaces. The freely diffusing studies allow one to investigate and resolve conformational populations in solution, while the surface tethered experiments allow one to resolve the individual folding and unfolding rate constants.

DNA, RNA Structure & Conformation II

2964-Pos Board B11
Equilibrium Bending Fluctuations of Short DNA
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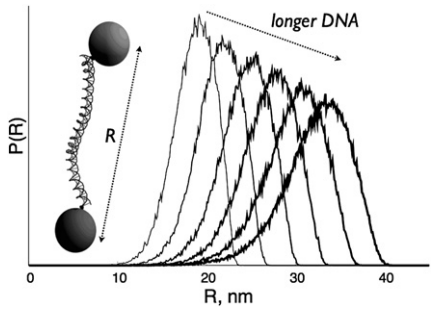
Short-lengthscale double-stranded DNA (dsDNA) bending elasticity plays an important role in many biological contexts. We measured dsDNA bending fluctuations using small-angle X-ray scattering from bulk solutions of two gold nanoparticles covalently linked by a 42-94 basepair dsDNA chain, freely fluctuating and not subject to external forces or surface interactions. This method reports on the equilibrium ensemble of bending fluctuations, not just an average value (as in ensemble FRET) or an extreme value (as in cyclization). Through Monte Carlo sampling of coarse-grained models, we find these experiments are consistent with a wormlike chain model of dsDNA, with a persistence length of ~50nm, in agreement with single-molecule force-extension experiments on much longer chains, but in contrast to recent suggestions of enhanced flexibility at these shorter lengthscales. These findings are plausible because even our shortest dsDNA chain still greatly exceeds the lengthscale of correlated microscopic fluctuations (roughly half a turn, 5 base pairs), and our observable is dominated by (high probability) mild fluctuations and thus insensitive to extremely rare fluctuations. Reproducing experiments required more detailed modeling at the DNA-gold interface, emphasizing the need for care in choosing the appropriate coarse-graining level for complex biomolecular systems.



2965-Pos Board B12
Re-Interpretation of Müller-Hill Experiments on the LacR-DNA Complex Using a Computational Model
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The looping of DNA by proteins such as the Lac repressor (LacR) is a fundamental gene regulatory mechanism. While our understanding of looping has certainly grown from advances in experimental and theoretical techniques, fundamental questions remain concerning the energetic cost of the reaction and the topology of the looped complex. In this study, we return to the well-established in vitro studies from the Müller-Hill lab concerning the LacR-induced looping of both linear and supercoiled DNA over a broad range of inter-operator lengths (6-21 helical turns). In these studies, gel electrophoresis was used to detect loop formation, to estimate loop size, and to quantify loop stability. For supercoiled DNA, gel electrophoresis also detected loop topology (Lk). Both DNase I protection experiments and electron microscopy further probed loop topologies. In returning to these experimental results, we use a new computational model to explore the experimental observations and to add new interpretations.