RNA Folding

2109-Pos Board B128
The Essential Adenosine Stacking in a Two-Base-Pair Minimal Kissing Complex
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A stable RNA helix requires at least three base pairs. Surprisingly, a tertiary kissing complex formed between two GAGC hairpin loops contains only two GC pairs. In the NMR structure of this complex, the two flanking adeninocles stack on the kissing GC pair. This observation raised a possibility that the 5'-dangling adenosines contribute to the formation and stability of the kissing interaction. To test this hypothesis, we took a two-pronged approach to examine the effects of various mutational and chemical modifications of the flanking adenosines on the folding of the kissing complex. Using mass spectrometry, we studied dimerization of various kissing hairpins. Using optical tweezers, we monitored mechanical unfolding of intramolecular kissing complexes at single-molecule level. In both experiments, replacing either adenine with a uridine abolished the kissing interaction, suggesting that a minimal kissing complex must contain two GC pairs linked by inter-strand stacking adenosines. The stabilizing effect by the adenosines can be explained by the fact that the stacking purine nucleobases shield the hydrogen bonds of the adjacent GC pairs, preventing them from fraying. Unlike in the context of secondary structure, the 5'-unpaired adenosines in the tertiary structure are structurally constrained in a way that allows for effective stacking onto the adjacent base pairs.

2110-Pos Board B129
The Role of Ionic Strength and Ion Valence in RNA Collapse and Folding
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RNA folds efficiently in the presence of divalent ions, Mg2+ ions in particular. In fact, enhanced charge screening by divalent ions is a recurring theme in the study of nucleic acid electrostatics. Although site-specific ions have been identified in RNA crystal structures, the counterion atmosphere that screens the RNA charge is largely composed of a diffuse and mobile ion cloud. Here, we use Small Angle X-ray Scattering (SAXS), Fluorescence Correlation Spectroscopy (FCS), and microfluidic mixing kinetics to investigate the role of ionic strength and ion valence in the interactions, collapse and folding of small model RNA molecules. Our measurements suggest that in the presence of divalent ions the effective charge density near the RNA surface is smaller. We propose that a tighter localization of divalent ions leads to a larger degree of charge compensation and facilitates RNA folding.

2111-Pos Board B130
A Novel Bent Intermediate in the Dimerization of HIV-1 DIS RNA
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Dimerization Initiation Sequence (DIS) plays a crucial role in genome dimerization through formation of a “kissing complex” intermediate between two homologous DIS sequences. DIS is a conserved hairpin loop motif on the 5′ UTR of the HIV-1 genome. This bimolecular kissing complex ultimately leads to the formation of an extended RNA duplex. Studying the mechanism and kinetics of this RNA interaction is the key to ex-plots DIS as a possible HIV drug target. Here, we report a novel study that makes an important contribution to understanding the dimerization mechanism of HIV-1 RNA in vitro. This work employed single-molecule fluorescence resonance energy transfer (smFRET) to monitor the dimerization of minimal HIV-1 RNA sequence containing DIS. Most significantly, we observe a previously uncharacterized folding intermediate that plays a critical role in the dimerization mechanism. Our data reveal that dimerization involves three distinct steps in dynamic equilibrium and the equilibrium between the steps are regulated by Mg2+ ions. Two of the steps are identified as previously proposed structures: the kissing complex and the extended duplex. In addition to these, our data reveal a previously unobserved folding intermediate, consistent with a bent kissing complex conformation, similar to the TAR-TAT complex. Mutations of the highly conserved purines flanking the DIS loop destabilize this intermediate which indicates that these purines may play an important role in the HIV-1 RNA dimerization in vivo. The mechanistic insights gained from these experiments would represent significant progress in understanding the HIV-1 dimerization mechanism.

2112-Pos Board B131
Global Studies of Single-Stranded Nucleic Acid Conformation
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Unstructured regions of RNA molecules require flexibility to accomplish many biological tasks such as conformational switching and protein recognition. Due to its highly charged backbone, the flexibility of single-stranded RNA is influenced by counterions. In this presentation, we continue to explore RNA flexibility using single-stranded nucleic acid homopolymers as a model system. We investigate the role of counterion valence in nucleic acid flexibility using a combination of small-angle X-ray scattering (SAXS) and single-molecule Förster resonance energy transfer (smFRET). We also study how charge-screening of these model systems are affected by mono- and divalent ions in competition. The results imply that various factors can alter the polymeric properties of unstructured nucleic acids, and may be important for tuning RNA conformational dynamics in vivo.


2113-Pos Board B132
RNA Folding Landscapes in the Presence of Putrescine and Magnesium
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RNA folding in vivo is driven by a diverse set of interactions with cytoplasmic components. Of these interactions, the screening of negatively charged phosphates on the RNA backbone by cations is critical for folding to the native structure. Given their high intracellular concentrations, the positive charges of Mg2+ and putrescine (totaling 100mM and 60mM respectively) neutralize a large fraction of negative charges possessed by structured RNA. Using an experiment analogous to equilibrium dialysis we observe the competitive interactions of putrescine and Mg2+ on an RNA ion atmosphere for both native and intermediate states of RNAs. These interactions are related to a folding free energy landscape for three different RNAs. In the presence of Mg2+, putrescine is found to be either stabilizing or destabilizing depending on the fold of the RNA. RNAs that chelate Mg2+ become destabilized in the presence of putrescine while non-chelators become more stabilized by putrescine. For some RNA molecules putrescine has an apparent synergistic effect on excess Mg2+, while other RNAs show an antagonistic effect. These results can be attributed to a difference in the manner in which putrescine and Mg2+ populate the ion atmosphere and affect RNA conformation.

2114-Pos Board B133
Structure and Functional Dynamics of Fluoride-Sensing Riboswitches
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Riboswitches are gene-regulatory RNA motifs located in the 5′ untranslated regions of certain bacterial mRNA’s. Riboswitches regulate gene expression by binding a metabolite related to the downstream gene, causing a conformational change that alters the accessibility of the gene for either transcription or translation. An important class of riboswitches that bind fluoride (F−) has been identified recently in bacteria and archaea, shedding light on how these organisms regulate internal fluoride concentrations to mitigate toxicity. The crystal structure of the fluoride riboswitch from Thermophilus petrophila shows a binding pocket in which the F− ion is coordinated by three Mg2+ ions. However, how ligand recognition and RNA folding are coupled to selectively encapsulate F− is not fully understood. Here, single-molecule TIRF microscopy and FRET are used to gain insights into the functional dynamics of fluoride riboswitches. Individual fluorescently-labelled fluoride riboswitches are immobilized on a quartz microscope slide, and the change in FRET efficiency between the fluorophors is used to study the ligand-binding mechanism and other cation- or denaturant-dependent structural transitions.

2115-Pos Board B134
Counterion-Dependent Folding of Azorarcus Ribozyme by SAXS Profile based Hybrid Simulation
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Multivalent counterions are essential for self-assembly processes of many functional RNAs. With increasing counterion concentrations, RNAs adopt compact intermediates and finally reach their native states. While the changes in global features such as molecular size and flexibility are often discussed using small angle X-ray scattering (SAXS) data, further details of the processes are difficult to address. Here, by sampling the conformational ensemble of the Azorarcus ribozyme using self-organized polymer model, we obtained the structural ensemble of the molecule that confirmed by a pair distance distribution function at various Mg$^{2+}$ ion concentrations. At each Mg$^{2+}$ ion condition, there could be many different structural ensembles, each of which equally reproduces the same SAXS profile. Hence, we carried out clustering analysis on the molecular ensembles by using the contact matrices defined for six different paired domains in secondary structure, and analyzed how the Azorarcus ribozyme reaches its native conformation by quantifying the similarity between the clusters. In consistent with the kinetic partitioning mechanism theory, the folding process of the Azorarcus ribozyme adopts multiple pathway. These pathways are more diverse above the transition mid-concentration of Mg$^{2+}$ ions, suggesting that there are many intermediates along the folding process. Interestingly, although urea shifts the Mg$^{2+}$ concentration of folding transition to a higher value, the dominant population of folding pathway is formed along the clusters with larger native contacts. This shows that urea can anneal the pathways of the Azorarcus folding dynamics towards the native state.

**2116-Pos Board B135 Combining Temperature and Force to Study Folding of Single RNA Molecules**

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Nucleic acids can be unfolded either by temperature, such as in UV melting, or by mechanical force using optical tweezers. In UV melting experiments, the folding free energy of nucleic acids at mesophotic temperatures are extrapolated from unfolding occurring at elevated temperatures. Additionally, single molecule unfolding experiments are typically performed only at room temperature, preventing calculation of changes in enthalpy and entropy. Here we present temperature controlled optical tweezers suitable for studying folding of single RNA molecules at physiological temperatures. Constant temperatures between 22°C and 42°C are maintained with an accuracy of 0.1°C, whereas the optical tweezers display a spatial resolution of ~1 nm over the temperature range. using this instrument, we measured the folding thermodynamics and kinetics of a 20-base-pair RNA hairpin by force-ramp and constant force experiments. Between 22°C and 42°C, the hairpin unfolds and refolds in a single step. Increasing temperature decreases the stability of the hairpin and thus decreases the force required to unfold it. The equilibrium force, at which unfolding and refolding rates are equal, drops ~1 pN as temperature increases every 5°C. At each temperature, the folding energy can be quantified by reversible work done to unfold the RNA and from the equilibrium constant at constant forces. Over the experimental temperature range, the folding free energy of the hairpin depends linearly on temperature, indicating that ΔH is constant. The measured folding thermodynamics are further compared with the nearest neighbor calculations using Turner’s parameters of nucleic acid folding energetics. Values of ΔS are comparable, however, ΔH from the two approaches are significantly different. The origin of such a difference will be further discussed.

**2117-Pos Board B136 Characterization of Viscous and Excluded Volume effects on the Folding Kinetics of the Tetraloop-Receptor Motif: Single Molecule Tests of Kramer’s Theory**

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It is widely known that there exists a strong relationship between the solvent, including co-solutes (e.g., cations, proteins, nucleic acids), and RNA that cooperatively defines the kinetics of tertiary structure formation. Viscosity, a solvent property that is coupled to dynamics, is critical in RNA folding due to the propensity for populating kinetically trapped species on a rough free energy landscape. Also of importance is the role of macromolecular co-solutes that limit the volume available for molecules to sample (excluded volume effects). The effects of these properties are highlighted by observed in vivo diffusion coefficients that are 4-200x smaller than in aqueous solvents. In this work, Kramers’ rate theory is used to describe the viscosity dependence of tetraloop-receptor docking kinetics at the single molecule level. Both rate constants, $k_{fold}$ and $k_{unfold}$ decrease with increasing viscosity (increasing glycerol %), trends that are predicted by Kramers’ theory in the over-damped limit. However, the same measurements made in high molecular weight PEG solutions showed folding rate constants are accelerated by 1-2 orders of magnitude. Scaled particle theory, describing a hard spheres PEG-RNA interaction, quantitatively predicts the stabilizing effect of excluded volume. Temperature dependent measurements show that the thermodynamics of docking are not perturbed (ΔH = −23(2) kcal/mol and ΔS = −76(6) cal/mol/K) even in up to 50% glycerol (6+P) and that solvent activation account for 4-6 kcal/mol of the folding enthalpy. In PEG solutions thermodynamics reveal that the folding is stabilized by a reduction in the entropy of the unfolded RNA ensemble (ΔS > 0). Thus, these studies validate physical models describing characteristic kinetic and thermodynamic trends for intramolecular structure formation attributed to viscosity and excluded volume.

**2118-Pos Board B137 Loop Length and Counter Ion Dependent Folding Kinetics and Thermodynamic Stability of Nucleic Acid Hairpin**

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Stem-loop structured hairpins represent major structural elements in nucleic acid. An intra-molecular double helix (stem) defines a DNA hairpin, usually stabilized by canonical base pairs or mis-pairs and capped by number of unpaired and paired nucleosides (loop). It is well known that nucleic acid hairpins play a pivotal role in DNA recombination, gene expression, and DNA transposition. A thorough investigation of kinetics and thermodynamic stability is the key to understand the role of DNA hairpin formation. It is well known that the structure and thermodynamic stability of DNA hairpin depend on stem size, loop size, stem and loop composition, base stacking, base-pairing, hydrogen bonds on the loop and closing base-pair of the loop. Here we use rapid-mixing stopped-flow kinetics and Fluorescence Correlation Spectroscopy (FCS) techniques to probe the loop length dependent folding kinetics and thermodynamic stability of DNA hairpins. Each hairpin is labeled with fluorophore Thioflavin T and quencher DABCYL at the 5' and 3' end respectively. FCS is a single molecule technique to measure reaction kinetics in the microsecond timescale where as stopped flow kinetics is a rapid mixing technique to monitor the reaction kinetics on the millisecond timescale. The reactions were observed to occur on a time scale of milliseconds by stopped-flow kinetics, considerably longer than ten of microseconds time scale suggested by previous kinetic studies of similar sized hairpins. Stopped flow kinetics and FCS studies show that nucleic acid folding and unfolding reactions occur over a broad range of timescales from the microsecond to milliseconds range. FCS can probe intermediate reaction and complete folding reaction can be probed by Stopped-flow kinetics. The combination of these two complementary techniques can probe the complete folding trajectory of the DNA hairpin.

**Virus Structure & Assembly**

**2119-Pos Board B138 Protein-Lipid Interactions in a Full-Scale Influenza a Virion: Insight into Flu Seasonality**

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The human influenza A virus exhibits seasonal infectivity in temperate regions, but there is still disagreement on the biophysical explanation. The recent publication of the lipidome of an influenza A virus provides compelling evidence that the virus buds from lipid rafts in the host plasma membrane, and that the virus is selectively enriched in cholesterol and sphingolipids beyond the plasma membrane raft from which it buds. These lipidomic details have allowed us to construct a full-scale computational model of the influenza A virion with a realistic lipid composition. We report preliminary findings on the lipid dynamics in these asymmetric viral envelopes containing the viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and the M2 ion channel, using coarse-grained molecular dynamics simulations. Furthermore, there is currently only indirect evidence for an interaction between the M1 matrix protein and the influenza A glycoproteins, resulting in a degree of uncertainty in the amount of mobile and restrained viral proteins. We have thus conducted simulations with both mobile and restrained viral proteins to assess the effect of viral protein mobility on the number, size and stability of lipid domains.