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 *Azoarcus* 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 *Azoarcus* 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 *Azoarcus* 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 *Azoarcus* folding dynamics towards the native state.

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Combining Temperature and Force to Study Folding of Single RNA Molecules

William T. Stephenson[1], Rachel Santiago[2], Sean Keller[2], Scott Tenenbaum[1], Michael Zuker[3], Pan T.X. Li[2].
[1]College of Nanoscale Science and Engineering, University at Albany - SUNY, Albany, NY, USA, [2]Department of Biological Sciences, University at Albany - SUNY, Albany, NY, USA, [3]Department of Mathematics, Rensselaer Polytechnic Institute, Troy, NY, USA.

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 mesophilic 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

Nicholas Dupuis, David J. Nesbitt.
JILA, Boulder, CO, USA.

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 ($\Delta H^\circ = -23(2)$ kcal/mol and $\Delta S^\circ = -76(6)$ cal/mol*K) even in up to 50% glycerol (~6 cP) and that solvent activation accounts 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 ($\Delta\Delta S^\circ > 0$). 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

Rajesh K. Nayak, Rebecca Faulkner, Olve B. Peersen, Alan K. Van Orden.
Colorado State Univertsity, Fort Collins, CO, USA.

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 TAMRA 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 tens 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 time scales from the microseconds 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

Tyler Reddy[1], Daniel L. Parton[2], Marc Baaden[3], Mark S.P. Sansom[1].
[1]SBCB Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom, [2]Department of Chemistry, University of Chicago, Chicago, IL, USA, [3]Laboratoire de Biochimie Theorique, Institut de Biologie Physico-Chimique, Paris, France.

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 motion expected of the 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.