An RNA kissing-loop from the Moloney Murine Leukemia Virus (MMLV) exhibits unusual mechanical stability despite having only two intermolecular base-pairs. Mutations at this junction have been shown to destabilize genome dimerization, with concomitant reductions in viral packaging efficiency and infectivity rates. Optical tweezers experiments have shown that it requires as much force to break the MMLV kissing-loop complex as is required to unfold an entire 11 base-pair RNA hairpin (Li PTX, Bustamante C, Tinoco I (2006) P.N.A.S. 103:15847). Using non-equilibrium all-atom molecular dynamics simulations, we have developed a detailed model for the kinetic intermediates of the force-induced dissociation of the MMLV kissing-loop. 208 dissociation events were simulated under constant applied external force, for a total of ~16 μs simulation time. These events are combined to construct a Markov state model for kissing-loop dissociation, which is capable of predicting overall dissociation rates on timescales longer than were actually simulated. We find that the complex undergoes a conformational rearrangement which distributes the applied force in parallel among multiple intermolecular hydrogen-bonds, which is substantially more mechanically stable than the serial bond breaking observed in the force-unfolding of RNA hairpins. Stacking interactions with adjacent, unpaired adenine bases further stabilize the complex by increasing the repair rates for partially broken base-pairs. These stacking interactions are also prominently featured in the transition state, which requires additional coordinates orthogonal to the end-to-end extension to be uniquely identified. Our findings can be extended to explain the peculiar sequence-stability relationships of the 6 base-pair HIV-1 DIS kissing-loop.

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Sub-Millisecond RNA Collapse Observed in a Microfluidic Mixer

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Measuring the fast collapse of RNA molecules can elucidate the first steps of RNA folding. The P5abc sub-domain of the Tetrahymena ribozyme is an example of an RNA molecule that folds rapidly following the addition of Mg2+ ions. We studied changes in truncated-P5abc (tP5abc) RNA as reported by Forster Resonance Energy Transfer (FRET) in a microfluidic mixing device using confocal microscopy. With sub-millisecond time resolution, we measured the ion species and valence dependent collapse timescales of tP5abc. In this presentation, we discuss differences between collapse times of tP5abc and those of a mutant which does not fully fold in the presence of magnesium ions. We also discuss experimental issues that arise when using an internally labeled fluorophore to report changes in RNA conformation. To account for changes in the FRET efficiency we consider results of fluorescence anisotropy measurements, as well as fluorescence correlation spectroscopy measurements.

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Cation Binding and Screening Effects on Single-Stranded Helix Formation in Poly(A) RNA

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The RNA homopolymer poly(A) forms single-stranded helices due to base-stacking, the mechanical properties of which may affect the biological function of naturally-occurring A-repeat sequences. Since RNA is a polyanion, screening and binding by cations enhances this helix formation by shielding charges along its backbone from each other.

The poly(A) single helix may be disrupted by application of tension, as by optical tweezers, allowing the energetics of helix formation to be estimated in addition to the mechanical properties of the helical and randomly-coiled states.

Using optical tweezers, we measured the force-extension behavior of poly(A) under a variety of buffer conditions, finding that the effect of sodium is consistent with Debye screening but that magnesium and other Group II cations are likely to be binding and forming inner-sphere (direct) contacts with poly(A), stabilizing the helix more than would be expected for screening alone. The mode of binding appears to be dependent on cation species, with distinct differences between binding by strontium and by magnesium and calcium.

To obtain quantitative information from these force-extension measurements, we have developed a new model for the force-extension behavior of poly(A) and similar helicogenic polymers, which unlike previous models takes into account Debye screening effects and the semiflexible nature of the helical state. Fitting this model to our data, we obtain estimates of helix formation energies and find evidence that magnesium binding distorts the poly(A) single helix into a mechanically distinct state.

3274-Pos Board B135

Riboswitch Folding Kinetics can Affect Gene Expression by a Translationally Acting Riboswitch

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In this research we study the thermodynamic and kinetic properties of translationally acting adenine riboswitches using single molecule spectroscopy. In general, adenine riboswitches are capable of switching conformations between states ''on'' (e.g., adenine bound aptamer folded) or ''off'' (e.g., repressor hairpin folded) for gene expression.

In our single molecule experiments we find that, around in vivo adenine concentrations, the ''on'' and ''off'' states can coexist in thermal equilibrium; whereas at low or saturating adenine concentrations, the equilibrium states are ''off'' or ''on'', respectively.

Importantly, however, we find long lifetimes in the range of tens of minutes for both the ''on'' and ''off'' states (i.e., lifetimes much longer than typical mRNA degradation times in bacteria) such that the riboswitch conformation may be effectively trapped. These long lifetimes for the ''on'' and ''off'' states are incompatible with cell cycle times, suggesting that the ''first fold'' in newly transcribed riboswitches could be an important determinant of translational control in these riboswitches.

3275-Pos Board B136

Single Molecule Views of the Ribosome Assembly

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Rapid and accurate assembly of the ribosome is a fundamental requisite for cellular functions. Ribosome assembly is a complex but highly selective and coordinated process of RNA folding induced/accompanied by binding of ribosomal proteins. Cryo-EM and footprinting studies have provided the hierarchical map of the assembly and molecular details of the folding pathways. Yet, the dynamics during ribosome assembly, despite their potential importance in selectivity and coordination, are not well understood. Using single molecule fluorescence resonance energy transfer, we could reveal an early phase of ribosome assembly dynamics by focusing on the 5' RNA domain of the small subunit. The RNA alone is highly dynamic and exhibits heterogeneous fluctuations. In particular, helix 3 undergoes extensive movement consistent with its switching mechanism proposed earlier based on footprinting data. Binding of the protein S4 reduces fluctuations but not completely, and binding of additional proteins further suppresses fluctuations and reduces heterogeneity as well. Interestingly, S4 protein binds to one conformation preferentially before switching to the other conformation that dominates in equilibrium, supporting the induced fit mechanism of S4 binding to RNA. Extending our approach to multiple colors should reveal the coordinated interplay of RNA folding and conformational dynamics with successive binding of proteins during ribosome assembly.

3276-Pos Board B137

Native Architecture Encodes Cooperativity and Specificity in RNA Folding Intermediates

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Structured RNA molecules are involved in numerous cellular processes that depend on the unique native structure. On the other hand, RNA chains are prone to misfolding due to their rugged energy landscapes. How do natural RNAs avoid local energy minima and fold specifically to the native conformation?

We addressed this problem by studying the role of native tertiary interactions in the folding of the Azoarcus group I ribozyme. Previous small angle X-ray scattering (SAXS) and pre-steady state activity showed that thermodynamic cooperativity between tertiary interactions emerges in folding intermediates, earlier than expected. Furthermore, these results indicated that native tertiary interactions in the core and periphery of the structure form concomitantly and suppress non-native folds.