Meanwhile, the single internal loop of 5BSL3.2 has been predicted to interact via kissing mechanism with a stem loop structure 200 nts upstream in the coding region and also with another stem loop (IIId domain) located in the 5'UTR of the HCV genome. In this study, we employ native gel electrophoresis and fluorescence spectroscopy to analyze simultaneously these multiple proposed kissing interactions. Our results provide direct experimental evidence for the kissing interactions among the CREs, suggesting a possible mechanism for their ability to function as molecular switches for essential steps in the HCV life cycle.

1291-Pos Board B201
Characterization of Supercoiled Double-Stranded RNA, and Comparison to Double-Stranded DNA
Gary M. Skinner, Serge P. Donkers, Jan Lipert, Zhuxiangdong Huang, Nynke H. Dekker

While the mechanical properties of double-stranded DNA have been intensely studied using various single molecule assays, much less is known about double-stranded RNA (dsRNA). dsRNA is important in Biology as it forms the hereditary material for a number of viruses, and is also central to the RNA mediated gene-silencing pathway. We have developed a novel “polynuclease-stall” labeling method for RNA that allows us to generate rotationally constrained molecules of dsRNA, an A-form helix. By anchoring these molecules to a coverslip at one end, and a magnetic bead at the other end, we are able to supercoil these molecules using a magnetic tweezer. We have mapped the elastic response across a range of forces and superhelical densities. In parallel we have performed identical measurements using rotationally constrained dsDNA, a B-form helix, of similar physical contour length. Our results show that for the most part, the two constrained nucleic acids respond in similar ways. For instance, we observe the denaturing of dsRNA when negative twist is added, at forces comparable to that of dsDNA. We have also quantified the slopes of the positive pleotonic regime, and these values are similar to dsDNA. However, we have discovered a striking difference between these two types of molecules at the buckling point, when positive twist is added. The kinetics of dsRNA buckling are much slower than for dsDNA, by several orders of magnitude. This observation implies that the activation energy for buckling dsRNA is much larger than for dsDNA, and we are currently seeking to understand the underlying physical reason for this.

1292-Pos Board B202
Structural Landmarks of the Hepatitis Delta Virus (HDV) Ribozyme
Kamali Sripathi, Pavel Banáš, Jiří Sponer, Michal Otyepka, Nils Walter

The hepatitis delta virus (HDV) is the only known human pathogen to contain a catalytic RNA motif (ribozyme) in its genome. The native structure of the HDV ribozyme consists of five helices (P1 - P4 and P1.1), which come together to form a double-nested pseudoknot. Within this complicated ribozyme are several landmines of structural importance, and alterations of these landmarks of ten lead to decreased ribozymal activity. We have used extended explicit solvent molecular dynamics simulations to investigate structural dynamics of HDV ribozyme. We plan on including effects of base protonation and base substitutions, using the available X-ray structures and their modifications as the starting structures. Specific attention has been paid to nucleotides in the catalytic pocket and the flexible L3 region. We have in detail investigated the effect of the force field choice on the ribozyme structural dynamics, by comparing several simulation force field variants, including our recent reparametrization of the chi profile of the Cornell et al (AMBER) force field.

1293-Pos Board B203
Tertiary Interactions Maintain the Balance of Stability, Folding Efficiency and Speed in a Large Catalytic Bacterial RNA
Reza Behrouzi, Joon Ho Roh, Duncan Kilburn, Robert M. Briber, Sarah A. Woodson

Like their protein counterparts, many non-coding RNAs, such as ribozymes, must fold into a unique 3D structure suited for their biological actions. These RNA structures are stabilized by an array of conserved tertiary interactions between residues far apart in sequence. However, the extent of thermodynamic coupling between tertiary interactions in RNA is little understood. To probe the basis for cooperative folding in RNA, we perturbed the folding energetics of a bacterial group I ribozyme with mutations that disrupt five conserved tertiary interaction sites. The assembly of core helices into compact native-like intermediates was measured by SAXS and native PAGE. Formation of the native state was probed with activity assays and hydroxyl radical footprinting of the RNA backbone. Although all of the mutants were catalytically active, their folding landscapes were significantly changed. Single mutations destabilized the compact intermediates and disrupted the thermodynamic link between tertiary interactions measured through double mutant cycles. Native PAGE and footprinting showed that loss of a single tertiary interaction affected the stability of other structural domains due to competition with non-native structures. Stepped-flow SAXS and hydroxyl radical footprinting with millisecond resolution showed that disruption of tertiary interactions does not hamper the rapid initial collapse of the RNA chain, but changes the folding hierarchy of the structural domains and the structures of populated intermediates. These results shed light on the interplay of the conserved tertiary interactions and electrostatic forces in the folding of large RNA molecules and delineate the mechanisms by which evolved RNA sequences determine the outcome of an otherwise nonspecific counterion-mediated collapse.

1294-Pos Board B204
Exploring the Folding Landscape of RNA in Crowded Solutions
Duncan Kilburn, Joon Ho Roh, Liang Guo, Robert M. Briber, Sarah A. Woodson

Crowder molecules in solution alter the configuration potential energy landscape of biological macromolecules. It is therefore critical to account for the influence of these other molecules when describing the folding of RNA inside the cell. Small angle x-ray scattering experiments were used to measure folding of a 64 kDa bacterial group I ribozyme in the presence of polyethylene-glycol with different molecular weights. We find that crowder molecules stabilize more compact states of the unfolded RNA, and also stabilize the folded state with respect to the unfolded state, as measured via the lowering of the folding midpoint on a MgCl2 titration. In addition, stopped-flow SAXS experiments with millisecond resolution show that the addition of crowder molecules speeds up the folding of RNA, even when the stability of the final folded state is held constant. These data indicate that crowder molecules change the folding landscape for RNA, allowing it to fold efficiently in Mg2+ concentrations that are well within the physiological range.

1295-Pos Board B205
The Role of Electrostatic Relaxation on the Folding Kinetics of a Bacterial Ribozyme
Joon Ho Roh, Robert M. Briber, Duncan Kilburn, Reza Behrouzi, Liang Guo, Sarah A. Woodson

The self-assembly of catalytic RNA ribozymes into compact, native structures is critical for their functions in the cell. The first step in forming RNA tertiary structure is the neutralization by cations of the negative charges of the phosphates. This electrostatic stabilization enables dynamical exploration of more compact conformations, and the search for long-range tertiary interactions. Our previous time-resolved Small Angle X-ray Scattering (SAXS) studies showed that the Azorarcus ribozyme exhibits triphasic folding kinetics: up to 90% folds in less than 10 ms, which we attribute to specific collapse. A second phase in ~40 ms is attributed to non-specific collapse followed by a conformational search for native structures. About 10% of the RNA folds in ~10 min, due to inability to sample misfolded ID RNAs. To examine how electrostatic interactions contribute to specific nucleation of the collapse transition, we used stopped-flow SAXS to monitor structural changes of the Azorarcus ribozyme over timescales ranging from 0.6 ms to ~10 seconds. Collapse and folding was induced by trivalent (Co2+, Spd2+), divalent (Mg2+ and Ba2+) and monovalent cations (Na+ and K+). The time-dependent decrease in Rg and disorder parameters show that pairing of the RNAs into the specific collapse pathway is greatest in Na+, while non-specific pathways are most populated in divalent cations. Interestingly, this change in the folding dynamics does not appear to originate from specific ion binding. Rather, the population of fast-folding RNA is directly proportional to the total number of cationic charges in excess of the number of cations required to half-stabilize the compact intermediate states. This result suggests that electrostatic relaxation plays a central role in the folding speed and specificity of the Azorarcus ribozyme.

1296-Pos Board B206
Structure of Deletion Mutant D5 RNA of a Group II Intron Ribozyme
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Group II introns are a class of self-catalytic ribozymes as well as mobile genetic elements found within the genes of all three kingdoms of life. They catalyze their own excision from pre-mRNA. It is hypothesized that pre-mRNA splicing may have evolved from group II introns due to the similar catalytic mechanism as well the structural similarity of the domain V substructure to the U6/2U extended snRNA of spliceosome. The secondary structure of group II introns is characterized by six typical stem-loop structures, also called D1 to D6. The domain V (D5) is a highly conserved Mg2+ binding platform and through extensive interactions with other intron domains, arranges the catalytic core for self-splicing. D5 is arranged into two helices separated by a bulge
The absence of the protein component ase P contains the enzyme active site and has the ability to process substrates in maturation of the 5' end of transfer RNA. In Bacteria, the RNase P holoenzyme Ribonuclease P is an essential enzyme that is responsible for catalyzing the

Lindsay E. Yandek
Structure
The Multiple Substrate Recognition Properties of Ribonuclease P: Achiev-
ing Uniformity in Processing Kinetics Despite Variation in Substrate Structure
Lindsay E. Yandek, Michael E. Harris.

Ribonuclease P is an essential enzyme that is responsible for catalyzing the maturation of the 5' end of transfer RNA. In Bacteria, the RNase P holoenzyme is composed of a RNA subunit and a protein subunit. The RNA subunit of RNase P contains the enzyme active site and has the ability to process substrates in the absence of the protein component in vitro, but works as a holoenzyme in vivo. While nucleotide recognition elements adjacent to the site of processing have been identified, different RNA sequences vary considerably. Remarkably, rates of pre-tRNA processing are uniform despite this variation in substrate sequence and structure. The mechanistic basis for multiple substrate recognition by the holoenzyme is the focus of this study, with the ultimate goal being a better understanding of uniformity in pRNA processing and discrimates between cognate and non-cognate RNAs. We are determining kinetic schemes for a number of pre-tRNAs using fluorescence assays and standard discontinuous assays. Multiple turnover kinetics have been obtained for pTRNA608, a consensus pre-tRNA, and pTRNA609, a non-consensus sequence. When pTRNA displays a similar Vmax and Km values, neither RNA displayed any burst nor lag phases in pre-steady state kinetics implying that the rate limiting step for our processing model is catalysis. Our initial hypothesis is that uniformity in substrate kcat/Km values results from differential 5' leader sequence interactions with the protein that compensate for deviations from tRNA consensus recognition sequences. To obtain more insight into sequence and structure influence, we have set up a series of multiple turnover experiments with a consensus and non-consensus pTRNA competing against one another.

Measuring the Dimensions of a Compact Kinetic Intermediate in the Folding Pathway of the glmS Ribozyme
Steve Meisburger, Krista Brooks, Suzette Pablt, Li Li, Joshua Blose, Ken Hampel, Lois Pollack.

Using complementary time-resolved biochemical and x-ray probes of RNA structure in solution, we investigate the cation-induced folding of the glmS ribozyme, a metabolite-sensing RNA switch that regulates gene expression in bacteria. Hydroxy radical footprinting experiments have shown a concerted folding transition within the first 30 seconds after adding magnesium. From small angle x-ray scattering (SAXS) experiments performed under similar conditions, we find that native tertiary contact formation is preceded by the collapse of the molecule to a relatively compact intermediate. The subsequent compaction observed by SAXS correlates temporally with changes in hydroxyl radical protection. We propose a structural model for the intermediate and possible implications for the role of secondary structure and electrostatics in the folding process of this ribozyme.

Folding Kinetics for the Conformational Switch Between Alternative RNA Structures
Song Cao, Boris Furtig, Harald Schwalbe, Shi-Jie Chen.

The conformational switching between different conformational states is intrinsic to RNA catalytic and regulatory functions, which often occurs on timescales of several seconds. In combination with the recent real-time NMR experiments (Weneter et al. Angew. Chem. Int. Ed. (2005), 44, 2600; Weneter et al. ChemBioChem. (2006). 7, 417) for the transitions between bi-stable RNA conformations, we combine the master equation method with the kinetic cluster method to investigate the detailed kinetic mechanism and the factors that govern the folding kinetics. Based on the computational studies, we propose that heat capacity change upon RNA folding may be important for RNA folding kinetics. In addition, we find that noncanonical (tertiary) intraloop interactions in tetraloop hairpins are important to determine the folding kinetics. Furthermore, through theory-experiment comparisons, we find that the different rate models for the fundamental steps (i.e., formation/disruption of a base pair or stack) can cause contrasting results in the theoretical predictions.

Site-Specific Labels to Study RNA Structure and Dynamics by NMR
Jacob N. Sama.

Ribonucleic acids are involved in many biological processes including catalysis, transfer and translation of genetic material, and regulation of gene expression. This unique ability to perform a variety of functions, traditionally associated with proteins is largely due to their capacity to adopt three-dimensional structural folds. Studying the 3D architecture of RNAs is critical not only for understanding the molecular basis of RNA function, but will eventually help with structure-assisted drug design, discovery and delivery. Heteronuclear NMR has become a powerful tool for studying the structure and dynamics of RNAs. To date, several RNAs have been well characterized by this method. However, overcrowding of chemical shifts and rapid signal loss in larger RNAs renders current NMR methods ineffective. To study the structure and dynamics of larger RNAs, the use of site-selectively 13C-labeled nucleotides promises to be very helpful. To synthesize these labels, it was hypothesized that the metabolic pathways of various Escherichia coli wild type and mutant strains are capable of producing specifically labeled nucleotides necessary for making RNA. To test this hypothesis, we evaluated the growth of mutant strains K10-1516 (deficient in glucose-6-phosphate dehydrogenase of the pentose phosphate pathway) and DL323 (deficient in the Krebs