thermodynamics of each type of kissing complexes one molecule at a time, and use mass spectrometry to take a snapshot of simultaneous equilibria of multiple kissing interactions by many molecules. Especially, we examine strength of relatively weak kissing interactions at single-molecule level and monitor their competition with formation of stronger kissing structures at ensemble level. To compare the two types of measurements, we take into account different experimental conditions, including salts, concentrations of RNAs, time vs. numerical averaging, equilibrium vs. non-equilibrium, and difference between intra- and intermolecular interactions. With these adjustments, we establish a quantitative correlation between two types of measurements, which can be used to accurately predict abundance of subpopulations, especially that of rare species, in a heterogeneous mixture. These results show that complementary information of an interacting network, generated by the two-pronged methodology, can be unified in a thermodynamic framework.

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Computational Analysis of Co-Transcriptional Riboswitch Folding

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Structured RNA in non-coding regions plays crucial regulatory roles in gene expression. Riboswitches are important representatives of such structured RNA that can prevent expression of the downstream gene by terminating transcription or attenuating translation. Typically, one out of two distinct structural conformations is formed depending on ligand binding. Transcription and extrusion of the nascent strand out of RNA polymerase take place at time scales comparable to those of folding and binding. We investigate these interdependent processes by simulating the extrusion out of RNAP and concurrent folding by two complementary computational techniques. Molecular Dynamics simulations with native structure-based models [1] provide atomically resolved structural detail while energetically more detailed kinetic Monte Carlo simulations [2] give access to longer timescales by describing folding on the secondary structure level. Depending on the scenario, we observe and quantify different pathways in structural formation which complements experimental measurements [3] and helps to understand the dynamic behavior of nascent RNA.

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Linking RNA Secondary Structure to the Free Energy of Tertiary Structure Folding through Coarse-Grained Models

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The importance of RNA secondary structure to 3D structure has long been appreciated, but a quantitative understanding of the linkage between the two has remained unclear. Recent results from our labs have indicated that simple steric and connectivity properties of RNA secondary structure strongly constrain and bias the 3D conformations that are sampled by RNA junctions. To further investigate the significance of these so-called topological constraints we have performed extensive simulations of various cytosolic tRNAs (ctRNA), mitochondrial tRNAs (mtRNA), and variants of the hairpin ribozyme using a specialized coarse-grained molecular dynamics model. This model reduces RNAs to collections of semi-rigid helices linked by freely rotatable single strands and ignores attractive and electrostatic forces, thus isolating the effects of topological constraints on RNA structure. Our simulations reveal that secondary structure changes as small as the insertion or deletion of one nucleotide into a junction loop can significantly alter the entropic cost of folding due to changes in topological constraints. In the hairpin ribozyme, such single nucleotide changes modulate the tertiary structure folding free energy by ~0.5 kcal/mol. More radical changes in junction topology alter folding free energies by >2 kcal/mol. These results are in strong agreement with previous experimental findings. In mtRNA, we show that a single nucleotide insertion, by altering topological constraints, causes a decrease in melting temperature of ~10° C. This decreased melting temperature provides a possible mechanism for the pathogenicity of such mutations in humans. Together, our results indicate that simple topological constraints imposed by secondary structure are a powerful determinant of RNA 3D structure.

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Coarse-Grain RNA Folding: Towards More Complex Structures

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RNA molecules are known to perform a variety of functions that are strictly dependent on their tertiary structures. Both large and small molecules can adopt complex and multiple architectures, including pseudoknots, multiple base pairs interactions, or alternative configurations as in riboswitches.
Despite experimental efforts, many questions remain on how RNA molecules fold and their thermodynamical properties. Similarly determining their tertiary structures is still a challenge by means of computer simulations.
With the aid of HiRE-RNA [1,2], a coarse-grained force field we develop, and enhanced sampling techniques such as Replica Exchange Molecular Dynamics [3], we are able to explore the conformational landscape of RNAs up to about 100 nucleotides, both in single and double strands, and to provide answers on both their dynamics and thermodynamics.
The introduction of a few constraints imposing a small number of base pairs, greatly improves the folding success by reducing the phase space. We discuss how these constraints can be introduced during simulation, and how the results depend on their numbers and locations in the RNA architectures.

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Observation of Global Changes in Conformation of an RNA Kissing Complex using Single-Molecular-Pair FRET

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We report on the observation of a change in the bend angle or twist of an RNA kissing complex upon Rop binding. Subtle global changes in molecular structure upon binding are generally difficult to discern using NMR or crystallography. FRET is well suited to observe these changes because of its sensitivity to interdye distance around the Förster radius, typically ≈5nm. For this reason, FRET is often referred to and used as a ''molecular ruler'' on this length scale. Here we show that for dye pairs that have minimal rotational freedom, FRET can also be used to observe changes in structure for which there is no significant change in distance between the dyes. The R1inv-R2inv kissing complex studied here is derived from the RNA I - RNA II system in E. coli. RNA II is a primer for replication of the ColE1 plasmid; its function is modulated by interaction with RNA I. Rop is known to bind and stabilize kissing complexes. It is also known to bind RNA in a structure, but not in a sequence dependent fashion. It has long been thought that Rop increases the bend of the R1inv-R2inv complex upon binding, but this has never been directly observed. Here we use FRET and modeling to investigate the structural change of this kissing complex upon Rop binding.

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Folding in Human Telomerase RNA Pseudoknots: Kinetic and Thermodynamic Studies via Single-Molecule FRET

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Telomerase is a ribonucleoprotein (RPN) responsible for maintaining the ends of linear eukaryotic chromosomes and has serious implications for both aging and cancer. The single RNA component of the RNP enzyme has a highly conserved pseudoknot motif that is critical for proper biological function. Single-Molecule Fluorescence Resonance Energy Transfer (smFRET) microscopy is used to investigate the folding kinetics and thermodynamics of a minimal wild-type (WT) pseudoknot construct. Urea is used in conjunction with the smFRET experiments to: (i) aid in determination of the kinetic and thermodynamics parameters