RNA Folding

2954-Pos Board B1

Kinetics and Mechanism of RNA Folding studied by SAXS

Joon Ho Roh[1,2], Reza Behrouzi[2], Robert M. Briber[1], Liang Guo[3], Dave Thirumalai[4], Sarah A. Woodson[2].
[1]Department of Materials Science and Engineering, University of Maryland, College Park, MD, USA, [2]T. C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD, USA, [3]Advanced Photon Source, Argonne National Laboratory, Argonne, IL, USA, [4]Institute For Physical Science and Technology, University of Maryland, College Park, MD, USA.

RNAs fold into unique native structures to perform specific biological functions. Detailed identification of the kinetics of RNA-folding should provide insight to the nucleation and collapse mechanisms. The *Azoarcus* ribozyme exhibits two thermodynamic transitions: 1) **U** to $\mathbf{I_c}$ at low Mg^{2+} concentrations (**U**: unfolded state, $\mathbf{I_c}$: compact intermediate state), ascribed to the assembly of double helices in the center of the RNA and 2) $\mathbf{I_c}$ to **N** at high Mg^{2+} concentration (**N**: native state), which involves further secondary-structural rearrangements to form the final folded structure including tertiary interactions. We have studied the rate of the folding transitions and the role of cooperative tertiary interactions in stabilizing the $\mathbf{I_c}$ and **N** states in order to understand the folding mechanism. The real-time dependence of folding of the wild type and mutant *Azoarcus* ribozyme as a function of cation concentration (Mg^{2+} or Ba^{2+}) was monitored using time-resolved small angle X-ray scattering (SAXS) coupled with a stopped-flow sample system. At high Mg^{2+} concentrations the *Azoarcus* ribozyme collapses through multiple pathways, with a majority (~ 90 %) following a fast folding route within $t \sim 10$ ms and the rest collapsing at $t > 3$ min. The folding rate at short time scales decreases with decreasing cation concentration. The rate changes significantly in the range of the first transition, suggesting that initial cation charge screening of the repulsion of the negatively charged RNA chains plays an important role in determining the formation of tertiary interactions. Interestingly, Ba^{2+} and single-site mutations in the *Azoarcus* ribozyme lead to faster collapse of the RNAs on short timescales, suggesting that folding of the wild type in Mg^{2+} requires more time to stabilize the correct folded tertiary structure through the rearrangement of secondary structures.

2955-Pos Board B2

Adaptive Seeding: A New Method for Simulating Biologically Relevant Timescales

Gregory R. Bowman, Xuhui Huang, Vijay S. Pande.
Stanford University, Stanford, CA, USA.

Molecular Dynamics (MD) simulations may complement experiments by allowing the evolution of molecular systems to be monitored with atomic resolution. However, it is currently only possible to simulate a few dozen nanoseconds of molecular dynamics in a month whereas most biologically relevant processes on the molecular scale take a few microseconds, or even seconds. Simulating a microsecond would take about a year so the utility of MD is limited. Our new adaptive seeding method, on the other hand, is able to completely sample processes that take tens of microseconds experimentally in a single week. This is accomplished by using short non-equilibrium simulations to identify the dominant thermodynamic states of the system. New simulations are then started from each state in a process called seeding and these simulations are found to give equilibrium sampling. We can then predict experimental observables like folding free energies and explain the chemical details underlying processes like folding. We have applied the adaptive seeding method to a small RNA system that folds in about ten microseconds. One set of simulations was started from the folded state determined by NMR while another was started from a random coil. The models obtained from each set of simulations agree within error and, therefore, represent the true equilibrium distribution of the molecule.

2956-Pos Board B3

Molecular Simulations of the Folding Kinetics of an RNA Tetraloop

Dong Guo, Angel E. Garcia.
Rensselaer Polytechnic Institute, Troy, NY, USA.

We study the folding kinetics of the r(GCUUCGGC) RNA tetraloop by combining molecular dynamics and replica exchange molecular dynamics simulations. An 11 microsecond REMD simulation with 52 replicas provides sufficient data to define the dominant conformations of the tetraloop. We use a clustering analysis method to identify these dominant states; both RMSD and Hamming Distance are used in the clustering. During the REMD simulation all replicas show multiple folding/unfolding events. The REMD trajectories along constant T segments of the simulations are combined with constant T MD simulations to obtain information about transition among states leading from the unfolded to the folded state. A model for the folding kinetics will be described.
This work is funded by the National Science Foundation MCB-0543769.

2957-Pos Board B4

How RNA Junctions Contribute To Folded Stability

Vincent B. Chu.
Stanford University, Stanford, CA, USA.

RNA Junctions are flexible regions that join helical elements of secondary structure in RNA. In this poster, we discuss how these elements may contribute to folded RNA stability by promoting tertiary contact formation.

2958-Pos Board B5

Predicting Structure And Stability For Simple H-type RNA Pseudoknots Involving Inter-helix Junction

Song Cao, **Shi-Jie Chen**.
University of Missouri-Columbia, Columbia, MO, USA.

A previous statistical mechanical theory predicted the structure and stability for canonical H-type RNA pseudoknot that contains no intervening junction between the helices (Cao and Chen 2006, Nucleic Acids Research, 34: 2634-2652). In the present study, we develop a model to treat H-type RNA pseudoknot involving a single-stranded junction between the helices. Specifically, we predict (a) the conformational entropy by explicitly consider the helix-helix, helix-loop, and loop-loop excluded volume interactions, and (b) the full free energy landscape from the RNA sequence. We find that loop-helix volume exclusion severely restricts the number of accessible loop conformations. Our extensive tests for the native structures from RNA sequences show good accuracy with overall sensitivity and specificity equal to 0.91 and 0.91, respectively.

2959-Pos Board B6

Bending and Internal Tension of an Intramolecular Kissing Complex

Pan T.X. Li.
University at Albnay, SUNY, Albany, NY, USA.

RNA tertiary base pairs are often bent and twisted by relative orientation of the interacting domains and by structures connecting these domains. Kissing complex, a basic RNA tertiary interaction, is formed by base pairing between distal hairpin loops. It remains unclear how much structure distortion a kissing complex can withstand. Crystal and NMR structures of several RNA kissing dimers show either a head-to-head coaxial helix or a slightly bent structure. An adenine riboswitch forms a well-bent two-base-pair kissing complex around its ligand. We designed two RNAs both consisting of a pair of hairpins connected by a single-strand. The two tetraloop hairpins in each RNA can form two GC kissing base pairs. The two RNAs, KC15 and KC30, have 15- and 30-nucleotide single-stranded linkers, respectively. Using optical tweezers, we examined mechanical folding of the two structures. With applied force, both RNAs can form an intramolecular kissing complex but show distinct mechanical unfolding patterns. Based on measured size and stability of the structures, we propose a mechanical model that such intramolecular kissing complexes, particularly the KC15, are bent to a blunt angle by internal tension of the single strand. Formation of such RNA triangle requires externally applied force by optical tweezers to offset the internal tension. Measurement of internal tension and structure distortion may facilitate understanding and prediction of tertiary interaction.

2960-Pos Board B7

Early RNA Folding Events During Assembly of the Signal Recognition Particle Monitored by Diffusion Single-Pair FRET

Goran Pljevaljcic, David P. Millar.
The Scripps Research Institute, La Jolla, CA, USA.

The Signal Recognition Particle (SRP) mediates the co-translational targeting of proteins destined for the secretory pathway. The particle recognizes a signal peptide as it emerges from the ribosome, temporarily halts translation and mediates docking of the SRP-ribosome-nascent chain complex to a membrane-bound SRP receptor. SRP consists of 7SL RNA and 6 SRP proteins. The ribonucleoprotein can be divided into two functional domains, the Alu domain and S domain. The last decade has yielded a significant amount of structural data on both of the SRP domains. However, dynamic information on the assembly and function of the SRP is lacking.
Here we present a diffusion single-pair FRET study to directly monitor early RNA folding events during SRP assembly. We investigated the detailed folding behavior of the Alu and S domain three-way junctions under the influence of both salt and SRP proteins. The Alu domain folds completely upon addition of either Mg-ions or the SRP9/14 heterodimer. This behavior indicates autonomous folding of the Alu RNA, with SRP9/14 acting only as cofactor to stabilize the folded RNA structure. In contrast, the S domain RNA folds only in the presence of the SRP19 protein, thus representing an example of protein-assisted folding. Hence, our studies of SRP assembly have provided examples of two basic mechanisms of RNA folding. Future studies will probe later folding events and the overall SRP assembly process.