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A New Free Energy Rule Toward Psuedo-knotted RNA Structure Prediction

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Accurate free energy estimation is essential for the prediction of RNA secondary structure. The widely Turner model works well for nested structures. For pseudoknotted RNAs, there is no effective free energy rules. We present a new free energy estimation method that goes beyond the Turner model based on the physical consideration that the free energy of loops (including pseudoknotted loops) are mostly determined by the loop length and the end-end distance. Given a specific secondary structure (refer to as topology hereafter), we grow the RNA in three dimensional space and search among all possible arrangement of helices for stable structure. The free energy contribution of helices is estimated from the Turner model, and the free energy of loops is calculated based on our new approach.

We assess our free energy model by testing its ability in selecting native structure from decoy topologies. For a testing set of 50-pseudoknotted RNAs, we create thousands of competitive decoy topologies and calculate their free energy. On average, our energy model provides an average of 7-8 percent improvement in term of sensitivity and specificity. In a blind pseudoknot structure prediction without inserting the native topology into the candidate list, our prediction has an average of 0.79 sensitives and 0.75 specificity. This performance is comparable to that of TdFOLD (0.76, 0.79) and better than Hotknots (0.69, 0.72), ILM(0.73, 0.69) and PKNOTS (0.78,0.73).

Our free energy estimation method is fundamentally different from the others: it is based on the growing of conformations in three dimensional space and is well suited for predicting pseudoknotted structures. In addition, our method also gives coarse 3D conformation of a given secondary structure, which can lead to accurate 3D ab initio predicted structure upon further refinement.

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An RNA Structural Ensemble Derived by Combining MD and Elongated NMR RDCs Provides Evidence for Adaptive Recognition via Conformational Selection

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We report implementation of a general strategy that combines molecular dynamics (MD) simulations and ''elongated'' NMR residual dipolar couplings for constructing atomic resolution dynamical ensembles of RNA with timescale sensitivity extending up to milliseconds. The ensemble of HIV-1 transactivation response element (TAR) constructed using this approach reveals spatially correlated inter-helical motions similar to those reported recently. Bulge residue U23 stacks onto A22, and together they undergo limited motions with U23 occassionaly looping out. Greater mobility is observed for the looped out bulge reidues C24 and U25. The corresponding ensemble obained for HIV-2 TAR in which bulge residue C24 is omitted, shows a significant reduction in the local mobility at A22, U23, and U25 which is accompanued by a reduction in the amplitude of inter-helical motions. The HIV-2 TAR ensmemble exhibits even more pronounced correlations between twisting motions around the two helices, indicating that these spatial correlations originate in part from having helices exert a pull on the other during to the finite length of the linker. The HIV-1 TAR ensemble contains conformers with local binding pockets and global inter-helical orientations similar to those observed in seven distinct ligand bound TAR conformations, supporting the notion that adaptive recognition occurs via 'conformational selection'.

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The Effect of Mg2+ on the Stability of two RNA Loop-Loop Complexes

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Mg2+ is particularly effective at stabilizing RNA tertiary structures. However, its mechanism of stabilization is not clearly understood. For instance, some tertiary structures do not form in the absence of Mg2+. In this work, we have studied two similar RNA complexes (''small complex'' and ''large complex'') in which two hairpin structures interact through their complementary loops. In both complexes, the loop sequences are identical but in the ''small complex'' the stems contain 5 base pairs while in the ''large complex'' the stems are larger. Previous studies have shown that the large complex does not form in the absence of Mg2+. The purpose of these experiments is to understand how the size of the stem affects the Mg2+ concentration required for complex formation.

Specifically, we used a combination of Circular Dichroism, UV melts and titration experiments to analyze the conditions under which these complexes form. Based on the hyperchromicity of our UV melts and the enthalpy of the unfolding transitions, we can say that the small complex forms in the presence of 250 mM NaCl or 5 mM MgCl2. Our titration experiments indicate that the small complex forms at Na+ concentrations as low as ~ 100 mM and Mg2+ concentrations of ~ 0.15 mM. The large complex also forms at ~0.15 mM Mg2+, consistent with their identical loops. However, the large complex does not form at low Na+ concentrations. Our CD experiments indicate that the large complex forms some structure at 400 mM NaCl but this structure is different than the Mg2+ induced loop-loop complex. The overall results suggest that the proximity of the phosphates in the loop-loop complex may create a specific site for Mg2+ while the presence of Na+ induces the formation of a slightly different complex.

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UV Melting and Footprinting Studies of Three Structures of an RNA Aptamer with the Same Sequence

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Using systematic evolution of ligands by exponential enrichment (SELEX), we have previously selected a class of competitive RNA aptamers against the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. One aptamer in this class was found to have a minimal, functional sequence of 58 nucleotides. In vitro transcription generates two RNA transcripts with the same sequence (M1 and M2), both of which are required to act together to inhibit the AMPA receptors. Although the M1 and M2 species have the same sequence, they cannot be inter-converted through the unfolding/refolding processes. To probe the secondary structures and the stabilities of these species, we carried out UV melting and RNA footprinting experiments. In these experiments, we used chemically synthesized AN58 (sAN58) as our control. sAN58 is a potent inhibitor by itself and possesses a different structure from either M1 or M2 (which are likely caused by co-transcriptional folding). Specifically, we have examined the effects of salt and urea on the melting profiles of the aptamers. All three RNA species had clearly distinct melting profiles with multiple melting transitions. M1, for instance, is found to have the highest melting temperature and the largest number of transitions. On the other hand, the melting profile of M2 is more similar to the sAN58 profile. These results suggest that all three RNA species have different structures, with M1 appearing to be the most stable of the three. Footprinting was also used to probe the secondary structure of the RNAs and to identify the nucleotides that interact with the S1S2 AMPA receptor ligand core.

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One RNA Aptamer Sequence, Two Structures: A Collaborating Pair that Inhibits AMPA Receptors

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GluR2 is one of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunits of the glutamate ion channel family. Excessive activity of the GluR2 AMPA receptor has been implicated in a number of neurological diseases, and therefore aptamers as inhibitors may be useful as pharmacological agents. Using systematic evolution of ligands by exponential enrichment (SELEX), we identified an aptamer, named as AN58, which competitively inhibits the AMPA receptor with nanomolar affinity. Surprisingly, the *in vitro* transcription of the DNA sequence of AN58 produces two stable RNA structures, M1 and M2, both of which are required for competitive inhibition of GluR2 AMPA receptors in a 1:1 stoichiometry and with roughly an equal affinity. Sequencing of M1 and M2 using primer extension dideoxy chain termination reaction suggests that they share indeed the same sequence. However, M1 and M2 are formed only co-transcriptionally. Once formed, they are not interconvertible through unfolding, such as boiling in ~50% formamide or NaOH treatment, and refolding. The results from reverse transcription reaction, in-line probing and chemical labeling experiment using *N*-methylisatoic anhydride (or SHAPE experiment) show that M1 and M2 have different structures. These findings indicate that the same DNA sequence can be transcribed into two RNA molecules that have the same sequence, but are structurally and functionally distinct. Thus, our results suggest more broadly that natural RNA molecules that show structural dissimilarities with different functions can nevertheless share a common ancestry and bear the same evolution memory.

Ribosomes & Translation

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Single-molecule Fluorescence Microscopy Study Of The Ribosome Translation Process

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