2977-Pos Board B24

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.

2978-Pos Board B25

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'.

2979-Pos Board B26

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.

2980-Pos Board B27

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.

2981-Pos Board B28

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

2982-Pos Board B29

Single-molecule Fluorescence Microscopy Study Of The Ribosome Translation Process

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The ribosome is the molecular motor responsible for the protein synthesis within all cells. Ribosome motions along the messenger RNA (mRNA) to read the genetic code are asynchronous and occur along multiple kinetic paths. Consequently, observation and manipulation at the single macromolecule level is desirable to unravel the complex dynamics involved. In this communication, we present the study of translation kinetics of single ribosomes via the direct observation of fluorescent amino-acid incorporations.

In order to study the kinetics of amino-acid incorporation inside the growing protein by the ribosome, we use a home-made total internal reflection single-molecule fluorescence microscope (TIRFM). The mRNA-ribosome complex is attached to a polyethylene glycol modified glass coverslip surface by a streptavidin-biotin linkage. The ribosome is labelled with a quantum dot (QD) in order to localize it on the surface while a specific amino acid (lysine) is marked with Bodipy-FL [1]. This fluorescent dye is small enough to enter the ribosomal channel thus leaving intact ribosomal activity. The protein synthesis is observed in real time as the labelled amino acids are incorporated into the polypeptidic chain by the co-localization of QD and Bodipy-FL fluorescence signals.

We will discuss the future application of this technique to single-molecule observation of the translation process, proof reading or even protein folding.

Reference

[1] K. Perronet, P. Bouyer, N. Westbrook, N. Soler, D. Fourmy, and S. Yoshizawa. Journal of Luminescence, 127, 264, 2007.

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How Initiation Factor 2 Regulates the Fidelity of Translation Initiation

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During prokaryotic translation initiation, the small, 30S and large, 50S ribosomal subunits, along with formylmethionyl-transfer RNA (fMet-tRNA^{fMet}), assemble at an authentic AUG messenger RNA (mRNA) start codon. The fidelity of initiation is regulated by three initiation factors (IFs) which kinetically control individual steps along the reaction pathway. One such step is recruitment of the 50S subunit to a 30S initiation complex (30SIC), composed of the 30S subunit, mRNA, tRNA, and IFs 1, 2, and 3. Although the GTPase IF2 has been shown to be principally responsible for selectively accelerating the rate of 50S subunit joining to a correctly initiated 30SIC, the molecular mechanism underlying this catalytic activity remains unknown. In order to elucidate the mechanism through which IF2 selectively catalyzes 50S subunit joining, we have developed single-molecule Förster resonance energy transfer (smFRET) signals between fluorescently-labeled IF2 and two tRNAs, an initiator fMet-tRNA^{fMet} and an elongator Phe-tRNA^{Phe}. Using these IF2-tRNA smFRET signals, we have characterized the dynamics of IF2-tRNA interactions within correctly and incorrectly initiated 30SICs. Our data reveal that the residence lifetime of IF2 on the 30SIC (τ_{30SIC}), a parameter that directly controls IF2's ability to recruit the 50S subunit to the 30SIC, is highly regulated during initiation. We find that τ_{30SIC} is exquisitely sensitive to: (1) the presence of IF1 and IF3, (2) the GTP- *vs.* GDP- or nucleotide-free forms of IF2, (3) the presence of fMet-tRNA^{fMet} *vs.* Phe-tRNA^{Phe}, and (4) the presence of a correct AUG start codon. Thus, only in the presence of IF1, IF3, GTP, and a correctly formed codon-anticodon interaction between an authentic AUG start codon and an initiator fMet-tRNA^{fMet} is IF2 binding to the 30SIC significantly stabilized such that 50S subunit joining is efficiently catalyzed, ensuring the fidelity of this step of translation initiation.

2984-Pos Board B31

Coupling of Ribosomal L1 Stalk and tRNA Dynamics during Translation Elongation

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Translation elongation necessarily requires large-scale movements of transfer RNAs (tRNAs) through the ribosome. While it is likely that these highly-coordinated tRNA movements are directed by conformational changes of the translating ribosome, data directly correlating ribosome and tRNA conformational dynamics are lacking. Using single-molecule ribosome-ribosome and ribosome-tRNA Förster resonance energy transfer (smFRET) signals, we have characterized the intrinsic conformational dynamics of the ribosomal L1 stalk as well as the coupling of L1 stalk and tRNA dynamics during translation elongation. We find that in post-translocation ribosomal complexes, the L1 stalk can occupy either an opened or closed conformation, with the probability and rate of transitions between these two states dependent on the occupancy and identity of the deacylated tRNA in the classical E site. Upon delivery of aminoacyl-tRNA into the ribosomal A site and peptide bond formation, however, a direct interaction between the closed L1 stalk and the newly deacylated tRNA residing in the hybrid P/E tRNA binding configuration is established spontaneously; this event involves coupled movements of the L1 stalk and the ribosome-bound tRNAs, as well as ratcheting of the ribosome. Our data reveal that the entire pre-translocation complex fluctuates between two global conformations: global state 1 (GS1) characterized by an open L1 stalk, classically-bound tRNAs and a non-ratcheted ribosome and global state 2 (GS2), characterized by a closed L1 stalk, hybrid-bound tRNAs, and a ratcheted ribosome. Binding of the ribosomal translocase, elongation factor G (EF-G), shifts the GS1/GS2 equilibrium towards GS2, promoting the intermolecular L1 stalk-tRNA interaction. Pre-steady state smFRET experiments reveal that the L1 stalk-tRNA interaction persists throughout the translocation reaction, suggesting that the L1 stalk allosterically collaborates with EF-G in order to direct tRNA movements during translocation.

2985-Pos Board B32

Dynamic Mode Switching and Loosely Coupled Conformational Events Observed in Single Ribosomes

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During protein synthesis transfer RNA (tRNA) and messenger RNA (mRNA) translocate through the ribosome in a rapid and stepwise fashion, a process that is catalyzed by the GTPase elongation factor G (EF-G). Single-molecule fluorescence resonance energy transfer (smFRET) measurements show that the ribosome complex can spontaneously adopt a key translocation intermediate in the absence of nucleotide hydrolysis. By obtaining smFRET data from multiple structural perspectives, we observe that tRNA and EF-G restructure the ribosome energy landscape, increasing the rate at which this intermediate is achieved. In particular, EF-G binding induces a fast dynamic mode of the L1 stalk, which facilitates rapid and reversible formation of the translocation intermediate. Bulk measurements indicate that the extent of conformational coupling is important in determining the rate of translocation. When the extent of coupling is increased, a faster rate of translocation is achieved. The rate of translocation is decreased when the conformational changes are further decoupled, such as by the addition of the antibiotic viomycin. Thus, the extent of coupling of conformational processes on the ribosome may present a new mechanism for the regulation of gene expression.

2986-Pos Board B33

An Allosteric Pathway Revealed in the Ribosome Binding Stress Factor BipA

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BipA is a highly conserved prokaryotic GTPase that functions as a master regulator of stress and virulence processes in bacteria. It is a member of the translational factor family of GTPases along with EF-G, IF-2 and LepA. Structural and biochemical data suggest that ribosome binding specificity for each member of this family lies in an effector domain. As with other bacterial GTPases, the ribosome binding and GTPase activities of this protein are tightly coupled. However, the mechanism by which this occurs is still unknown. A series of experiments have been designed to probe structural features of the protein to see if we can pinpoint specific areas of BipA, perhaps even individual residues, which are important to its association with the ribosome. Included in the list are the C-terminal effector domain of the protein, which is distinct to the BipA family of proteins, and amino acid residues in the switch I and II regions of the G domain. Using sucrose density gradients, we have shown that the C-terminal domain is required in order for BipA to bind to the ribosome. Moreover, deletion of this domain increases the GTP hydrolysis rates of the protein, likely through relief of inhibitory contacts. Additional evidence has revealed an allosteric connection between the conformationally flexible switch II region and the C-terminal domain of BipA. Site directed mutagenesis, sucrose gradients and malachite green assays are being used to elucidate the details of this coupling.

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Rare Codon Clustering: Implications for Protein Biogenesis

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Most amino acids are encoded by more than one codon. These synonymous codons are not used with equal frequency: in every organism, some codons are used more commonly, while others are more rare. Though the encoded protein sequence is identical, selective pressures favor more common codons for enhanced translation speed and fidelity. However, rare codons persist, presumably