2444-Pos
Single-Molecule Analysis of Mss116-Mediated Group II Intron Folding
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Group II introns are one of the largest self-splicing ribozymes found in organellar genomes of various eukaryotes such as fungi, plants and some animals as well as in bacterial genomes. Interestingly, these ribozymes can self-splice via two transesterification reactions that resemble nuclear pre-mRNA splicing in eukaryotes as they share some characteristic features with spliceosomal introns. Despite the diversity in primary sequences, group II introns show highly conserved secondary structure that consists of six domains (D1-D6) radiating from a central core. Out of six domains, D1 and D5 are the only domains absolutely essential for minimal catalytic activity, but the presence of D2 and D3 enhances catalytic activity of the intron. In order to perform its functions, large multidomain group II intron RNA must adopt the correctly folded structure. As a result, in vitro splicing of group II introns requires high ionic strength and elevated temperatures. In vivo, this process is mainly assisted by protein cofactors. However, the exact mechanism of protein-mediated splicing of group II intron RNA is still debatable.
In order to understand the mechanism of protein-mediated splicing of group II introns, we studied the folding dynamics of D135 ribozyme, a minimal active form of the yeast ai5γ group II intron, in the presence of its natural cofactor, the DEAD-box protein Mss116, using single-molecule fluorescence. Based on our single-molecule FRET data, Mss116 together with ATP facilitate the formation of the folded native state mainly by stabilizing on-pathway intermediates.

2445-Pos
Time-Resolved Multiphase Folding of Bacterial Group I Ribozyme
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Understanding of microscopic mechanism of the RNA folding process is critical for mapping out the complex mechanisms by which RNA mediates various cellular events including gene-information transfer and catalysis. Folding of RNA occurs in the presence of cations that neutralize the anionic charge of the phosphate groups, and subsequently enable long distance tertiary interactions between nucleotide bases. The process by which RNA rapidly finds its unique three-dimensional shape despite countless possible conformations remains an unsolved problem. We aim to understand the roles of electrostatic neutralization and cooperative tertiary interactions in the kinetic nucleation of structure and collapse. We have used time-resolved small angle X-ray scattering (SAXS) coupled with a stopped-flow sample system that covers sub-ms timescales to monitor the real-time structural change in the *Azoarcus* ribozyme. The collapse process was found to have at least two distinct kinetic phases within 100 ms. As the concentration of cations increases, both the kinetic constant (k) and fraction of fast collapsing phase increase up to $k \sim \geq 3000\ s^{-1}$ and 80 % for high Mg^{2+} concentrations and from ~ 2000 s^{-1} and 40 % for low Mg^{2+} concentrations. This suggests that the initial charge-screening rate is crucial for determining not only the kinetic constant for the formation of tertiary interactions but also the partitioning into faster pathways. A minority (~10%) of the *Azoarcus* ribozyme falls into slow collapsing pathways, with $k \sim 0.03\ s^{-1}$. The results imply that the multiphase collapse process originates from an ensemble of heterogeneous structures folding on different pathways rather than a sequential step-like process. Strong kinetic heterogeneity of the collapse nucleation appeared near the thermodynamic transition, which suggests a strong competition among energetically different collapse pathways.

2446-Pos
Folding of Bacterial Group I Ribozyme in Crowded Solutions
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Crowder molecules in solution alter the equilibrium between folded and unfolded states 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, which contains up to 30% by volume of macromolecules.
In this contribution we report the results of small angle x-ray scattering experiments on a catalytic RNA, or ribozyme, from the bacterium Azoarcus sp BH72 in the presence of polyethylene-glycol 1000 (PEG-1000), a molecular crowder with average molecular weight 1000 Da. We observe that, in agreement with expected excluded volume effects, the presence of PEG shifts the equilibrium to favor more compact RNA structures. Two observations highlight the stabilization of the compact RNA: firstly, the transition from the unfolded to folded (more compact) state occurs at lower MgCl2 concentrations in PEG; secondly, the radius of gyration of the unfolded RNA decreases from 76 Å to 64 Å as the PEG concentration increases from 0 to 20 % wt./vol.
We discuss several physical effects by which the crowder molecules can possibly influence the RNA folding. These include modification of ionic activity, modification of water activity, and the excluded volume effect. We present evidence that whilst all of the above effects probably change the RNA folding with respect to a dilute aqueous solution, the shift in Mg2+ dependence of folding transition cannot be explained entirely by the change in ion activity. We conclude that the dominant influence is likely to be the excluded volume effect.

2447-Pos
Hepatitis C Virus (HCV) - 3′UTR : a Kissing Complex Dependent Molecular Switch ?
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With over 200 milllion people infected with the highly mutational hepatitis C virus (HCV), the need to find a conserved and functionally essential therapeutic target within the HCV genome is imperative. The 3'UTR of the HCV RNA genome, contains a 100% conserved 55 nucleotide (nt) sequence which has been proposed to be involved in interactions with a conserved sequence located within the HCV RNA coding region, interactions essential for HCV replication. Interestingly, the same 55 nt region has also been shown to be involved in the HCV genome dimerization through an unknown mechanism. In this study, we used different biophysical techniques to elucidate that the HCV RNA dimerization occurs through a kissing complex intermediate which is converted to a more stable duplex in the presence of the HCV core protein. We also provide for the first time direct experimental evidence for the interactions of the 3'UTR 55nt with the coding region of the HCV genome. Our results, which reveal dual ability of the HCV 3'UTR region to interact with both, the conserved HCV RNA sequence in the coding region, as well as with another HCV RNA genome, strongly suggest that the 3'UTR might serve as a molecular switch within the HCV life cycle for processes such as viral replication and packaging. Thus this region could serve as an extremely potential therapeutic target, allowing the inhibition of more than one process in the HCV life cycle.

2448-Pos
The Statistical Properties of Human UTRs Compared to that of Random Sequences
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Untranslated Regions (UTRs) are ubiquitous controllers of genetic networks and are a key component of the cell's regulatory machinery. The molecular mechanism by which these UTRs achieve their regulatory efficacy is still poorly understood. We postulate that the RNA structure of these UTRs plays a central role in regulation. We compare the statistical properties of over 21,000 5' and 3' UTRs from the human genome of less then 1,000 nucleotides to two sets of randomly generated sequences. One set of random sequences the sequences of the human UTRs are scrambled to keep the same distribution of GC content and length. The second set of random sequences contains 1,000 completely random sequences for each combination of GC content from 0 to 1 and lengths from 25 nucleotides to 1,000 nucleotides. The scrambled sequences allow us to compare distributions of the statistical properties such as the Shannon entropy and the Helix entropy of the RNA partition function. The second set of random sequences allowed us to obtain empirical rules for predicting the average properties such as number of bases paired and average helix length of a sequence given the GC content and the length. With these comparisons it is possible to make predictions about the resilience of a given UTR to mutation and how stable a particular UTR is compared to a random sequence.

2449-Pos
Assembly Mechanisms of RNA Pseudoknots are Determined by the Stabilities of the Constituent Secondary Structures
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Understanding how RNA molecules navigate their rugged folding landscapes holds the key to describing their roles in a variety of cellular functions. To dissect RNA folding at the molecular level, we performed simulations of three pseudoknots (MMTV and SRV-1 from viral genomes and the hTR pseudoknot from human telomerase) using coarse-grained models. The melting temperatures from the specific heat profiles are in good agreement with the available

experimental data for MMTV and hTR. The equilibrium free energy profiles, which predict the structural transitions that occur at each melting temperature, are used to propose that the relative stabilities of the isolated helices control their folding mechanisms. Kinetic simulations, which corroborate the inferences drawn from the free energy profiles, show that MMTV folds by a hierarchical mechanism with parallel paths i.e., formation of one of the helices nucleates the assembly of the rest of the structure. The SRV-1 pseudoknot folds in a highly cooperative manner and assembles in a single step in which the preformed helices coalesce nearly simultaneously to form the tertiary structure. Folding occurs by multiple pathways in the hTR pseudoknot, whose isolated structural elements have similar stabilities. In one of the paths, tertiary interactions are established prior to the formation of the secondary structures. Our work shows that there are significant sequence-dependent variations in the folding landscapes of RNA molecules with similar fold. We also establish that assembly mechanisms can be predicted using the stabilities of the isolated secondary structures.

2450-Pos
Mechanical Folding Kinetics of RNA Hairpins: a New Computational Approach
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From the distribution of the low-lying states on the energy landscape, we recently developed a new computational method for the prediction of RNA folding kinetics [1]. The method can treat long sequences because it is based on a small ensemble of seed states. Additionally, the method is based on an analytic formalism and thus enables predictions for long-time folding kinetics. Applications of the new kinetic model to mechanical folding of RNA hairpins reveal distinct kinetic behaviors for a wide range of different force regimes, from zero force to forces much stronger than the critical force for the folding-unfolding transition. In the low force limit, folding can be initiated (nucleated) at any position by the formation of the first base stack and folding can proceed through many parallel pathways. In contrast, for a higher force, the folding/unfolding would predominantly proceed along a single zipping/unzipping pathway. Studies for different hairpin-forming sequences indicate that depending on the nucleotide sequence, a kinetic intermediate can emerge in the low force regime but disappear in high force regime, and a new kinetic intermediate, which is absent in the low and high force regimes, can emerge in the medium force range. Variations of the force lead to changes in folding cooperativity and rate-limiting steps. For TAR RNA sequence, we predicted two parallel dominant pathways. The rate-limiting folding steps (at $f = 8$ pN) are the formation of specific base pairs that are 2-4 base pairs away from the loop. At a higher force ($f = 11$ pN), the folding rate is controlled by the formation of the bulge loop. The predicted rates and transition states are in good agreement with the experimental data for a broad force regime.
[1] Cao and Chen, 2009, *Biophys. J.*, **96**, 4024-4034.

2451-Pos
First-Principles Prediction of the Sequence-Dependent Stability of RNA Hairpin Loop
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We develop a statistical mechanical model to predict the sequence-dependent folding stability from the sequence for simple RNA hairpin loops. We use a recently developed 3-vector virtual bond-based RNA folding model which enables rigorous computation of RNA chain entropy. Enumeration of all the possible arrangements of base pairs and the corresponding conformational entropies, through exhaustive self-avoiding random walks of the virtual bonds in a diamond lattice, gives the partition function and free energy for a given RNA sequence. The new model developed here has two unique advantages. First, the model is based on the complete conformational ensemble, including loop conformations that contain all the possible intra-loop base pairs and/or terminal mismatches. Therefore, intra-loop base pairs and mismatches are the results predicted from the model instead of known input information for the model. Second, the model gives accurate estimation for the dramatic entropic changes caused by the formation of the intra-loop base pairs and mismatches. Our first principles calculations for the chain entropy for each given set of base pairs, in combination with the empirical energy function provided from Turner rules, provide ab initio predictions for the sequence-dependent loop stability from RNA sequence. Tests against experimental data indicate that the theory can give improved predictions for the sequence-dependent RNA hairpin loop stability than the nearest-neighbor model.

2452-Pos
Salt Dependent Folding Energy Landscape of RNA Three-Way Junction
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RNAs are highly negatively charged molecules. Salt ions are crucial for RNA folding stability and conformational changes. In the present work, we employ the recently developed tightly bound ion (TBI) model, which accounts for the inter-ion correlations and the fluctuation of ion distributions, to investigate the ion-dependent free energy landscape for the three-way RNA junction in a 16S rRNA domain. The predicted electrostatic free energy landscape suggests that (a) ion-mediated electrostatic interactions cause an ensemble of unfolded conformations narrowly populated around the maximally extended structure and (b) Mg2+ ion-induced correlation effect may help bring the helices to the folded state. Non-electrostatic interactions, such as non-canonical interactions within the junctions and between junctions and helix stems, might further limit the conformational diversity of the unfolded state, resulting in a more ordered unfolded state than the one predicted from the electrostatic effect. Moreover, the folded state is predominantly stabilized by the coaxial stacking force. The TBI-predicted folding stability agrees well with the experimental measurements for the different Na+ and Mg2+ ion concentrations. For Mg2+ solutions, the TBI model, which accounts for the ion correlation effect, gives more accurate predictions than the Poisson-Boltzmann theory, which tends to underestimate the role of Mg2+ in stabilizing the folded structure. Detailed control tests in-dicate that the dominant ion correlation effect comes from the charge-charge Coulombic correlation rather than the excluded volume (size) correlation between the ions. Furthermore, the model gives quantitative predictions for the ion size effect in the folding stability.

Chromatin

2453-Pos
Diffusion Based Looping of Chromatin
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Chromatin folding inside the interphase nucleus of eukaryotic cells is done on multiple scales of length and time. Despite recent progress in understanding the folding motifs of chromatin, the higher-order folding still remains elusive. Flourescent in situ hybridization reveals a tight connection between genome folding and function as well as a folding into a confined sub-space of the nucleus. The folding state of chromatin reveals distinct differences from a compact conformation. A previously published model, the random loop (RL) model, explains the folding state by the formation of random loops, which themselves seem to be an ubiquitous motif of transcriptional regulation. However, it remains a crucial question what mechanisms are necessary to make two chromatin regions become co-located, i.e. have them in spatial proximity.
The model presented here bridges the gap between statistical polymer models and an effective description of the dynamic process of loop formation mediated by the nuclear environment. Without assuming long-range forces or any active transport mechanisms, this model assumes that the formation of contacts or loops is done solely on the basis of random collisions. The probabilistic nature of the formation of temporary contacts mimics the effect of e.g. transcription factors in the solvent. Although only basic interactions are taken into account, this model is in agreement with recent experimental data.

2454-Pos
Prokaryotic Chromosome Organization in the Context of Entropy, Confinement and Tethering Interactions
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Prokaryotic chromosomes are physically organized and condensed into an intricately structured DNA-protein complex called a nucleoid. The large-scale physical structure might arise from protein mediated interactions that can form both inter and intra-chromosome tethers as well as anchoring the chromosome to the membrane of the nucleoid or to protein scaffolds [1]. Motivated by recent experiments that capture E. coli nucleoid structure using three spectrally distinct, fluorescently-labeled genetic loci [2], we analyze single-locus and two-locus positioning distributions in the theoretical framework of a coarse-grained polymer model taking into account excluded volume, confined geometries as well as tethering interactions therewith shedding light into the mechanisms governing E.coli nucleoid structure between replication cycles.
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