Board B80.05

Trigger Factor (TF) is an important catalyst of nascent peptide folding and possesses both peptidyl-prolyl *cis-trans* isomerase (PPIase) and chaperone activities. TF has a modular structure, containing three domains with distinct structural and functional properties. The N-terminal domain of TF is important for ribosome binding and the M domain carries the PPIase activity. However, the function of the C-terminal domain remains unclear and the residues or regions directly involved in substrate binding have not yet been identified. In our previous studies we have obtained experimental evidence that TF is effective as a molecular chaperone and its chaperone activity is distinct from its PPIase activity both *in vitro* and *in vivo*. Here, guanidine induced equilibrium and kinetic folding of a variant of green fluorescent protein (F99S/M153T/V163A, GFPuv) was studied. Using manual mixing and stopped-flow techniques, we combined different probes, including tryptophan fluorescence, chromophore fluorescence and reactivity with DTNB, to trace the spontaneous and TF-assisted folding of guanidine denatured GFPuv. We found that both unfolding and refolding of GFPuv occurred in a stepwise manner and a stable intermediate was populated under equilibrium conditions. The thermodynamic parameters obtained show that the intermediate state of GFPuv is quite compact compared to the denatured state and most of the green fluorescence is retained in this state. By studying GFPuv folding assisted by TF and a number of TF mutants, we found that wild-type TF catalyzes proline isomerization and accelerates the folding rate at low TF concentrations, but retards GFPuv folding and decelerates the folding rate at high TF concentrations. This reflects the two activities of TF, as an enzyme and as a chaperone. A general mechanism of TF assisted protein folding is discussed..

1104.06-Pos Copolymer Surfactant-Assisted Disaggregation of Denatured Proteins depends on Surfactant

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Board B80.06

Intracellular accumulation of misfolded or unfolded proteins is a well established as a part of the pathogenesis of certain human diseases, particularly thermal injury and neurodegenerative conditions. Effective therapies to refold or eliminate these proteins are needed. Certain biocompatible multiblock copolymer surfactants are capable of disaggregating and refolding denatured proteins. This research project was designed to investigate, using Rayleigh light scattering methods, whether disaggregation and refolding of heat denatured proteins was dependent on the structure of the surfactant, protein or both. We examined the refolding of two thermally denatured proteins: lysozyme and bovine serum albumin (BSA) in response to three different surfactants: Poloxamer 108 (P108), Poloxamer 188 (P188), and Tetronic 1107 (T1107). We observed that these three surfactants are capable of reducing the size of denatured protein aggregates and T1107 was more effective than the smaller triblock surfactants. By comparison, we observed that equimolar 10 kDa polyethylene glycol (PEG), a monoblock hydrophilic polymer, was incapable of disaggregating these proteins. Finally, we observed that surfactant disaggregation of chemically denatured Carboxypeptidase A restores its catalytic activity. These findings indicate that amphiphilic surfactant copolymers are efficient in disaggregating proteins and are more effective than hydrophilic polymers.

RNA Folding

1105-Pos Nucleotide Analog Interference Mapping of Ligand Mediated Conformational Changes in the SAM Riboswitch

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Riboswitches are RNA genetic regulatory elements. Upon binding a small molecules, such as a metabolite, a conformational change occurs that either promotes or precludes continued transcription or translation. Here we investigate the mechanism of structural stabilization by S-adenosylmethionine in the SAM I riboswitch using NAIM(Nucleotide Analog Interference Mapping). The results with A and G analogs suggest the conformational change to the SAM bound form is related to pseudoknot formation.

1106-Pos Two-dimensional Infrared Spectroscopy Of RNA Folding

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The phenomenon of RNA folding has been studied with a variety of methods, including molecular dynamics simulations, temperature jump kinetics, and NMR spectroscopy. Many questions remain unanswered, including how base pairing and base stacking interactions influence folding and what mechanism the folding follows. These interactions have characteristic vibrational signatures that can be used to provide detailed structural information about RNA strands during folding events. In this study, we simulate the 2DIR signal of several RNA motifs along their folding trajectories. Novel pulse configurations are used to improve the resolution of cross-peaks. By following the evolution of the spectra as the RNA unfolds, we are able to see how the coupling interactions change. The different model strands have distinct spectra, making 2DIR a powerful tool for more detailed structural analysis of RNA folding. We use our results to propose possible folding pathways and discuss the use of more detailed models to describe the coupling interactions in the future.

1107-Pos Predicting the Sizes of Large RNA Molecules

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A great deal of work has been done to determine the structure of small (say, hundred-nucleotide-long) single-stranded (ss) RNA molecules, particularly those with enzymatic activity. On the other hand, there are dramatically fewer studies of very large ssRNA molecules (say, several thousands of nucleotides), particularly viral RNA. Viral RNA is special because it is frequently as long as 10,000 nucleotides, and because it must fit into a very small volume (20 – 30 nm in diameter) to be protected by the viral protein shell. This raises immediately the following question: How big is a large ssRNA molecule? In particular, what is the overall, three-dimensional (3D) size - e.g., the radius of gyration (R_g) - and how does it depend on the number *and sequence* of nucleotides? We argue that there are certain coarse-grained properties of the secondary structures of large ssRNAs that can be reliably predicted and that provide useful measures of the 3D sizes of these molecules. We introduce a quantity called the *maximum ladder distance* (MLD) and show that it can distinguish between viral and nonviral nucleotide sequences of the same length; the viral MLDs are consistently and significantly smaller, independent of the particular algorithm chosen to determine secondary structure. In addition, we argue that the MLD - an average, large-scale property of sequence-dependent secondary structure - provides a useful measure of the relative 3D sizes of large ssRNAs, according to the scaling relation $R_g \sim MLD^{1/2}$. Finally, we conjecture that the overall *many*-gene nucleotide sequence is under evolutionary pressure to give rise to a global secondary structure that results in a 3D size compatible with the capsid into which it must fit.

1108-Pos Direct Observation of Hierarchical Folding in Single Riboswitch Aptamers

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Riboswitches regulate gene expression through structural changes in ligand-binding RNA aptamers. Using an optical-trapping assay based on *in situ* transcription by a molecule of RNA polymerase, single RNA transcripts containing *pbuE* adenine riboswitch aptamers were unfolded and refolded. Multiple folding states were characterized using force-extension curves (FECs) and folding trajectories under constant force by measuring the molecular contour length, kinetics, and energetics, both with and without adenine. Specifically, the aptamer became more resistant to force disruption upon binding adenine, generating larger unfolding forces in FECs. The kinetics of aptamer folding and adenine binding were investigated using this mechanical signal; after varying lengths of refolding time and at multiple adenine concentrations, the aptamer structure was probed and the adenine-bound fraction fit to a three-state kinetic model, which furnished both the timescale for folding of the aptamer to a state competent to bind adenine and that for binding of adenine. Under physiological conditions, both of these timescales are comparable to the regulatory timeframe of complete riboswitch transcription. In addition, measured unfolding forces for many FECs taken from a single molecule were analyzed using recently-developed methods for extracting kinetic and energetic parameters from non-equilibrium unfolding experiments. Finally, constant-force folding trajectories at successively decreasing force revealed stepwise folding of the aptamer, from which a complete energy landscape for aptamer folding in the presence and absence of adenine was created. Distinct folding steps were correlated with the formation of key secondary and tertiary elements as well as with ligand binding. Importantly, the adenine-induced stabilization of the weakest helix in the aptamer, the mechanical switch underlying regulatory action, was observed directly. These results provide an integrated view of hierarchical folding in an aptamer, demonstrating how complex folding can be resolved into constituent parts.

1109-Pos Single-Molecule Mechanical Unfolding and Folding of a Pseudoknot in Human Telomerase RNA

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Mechanical stretching forces perturb the RNA folding landscape by favoring the more extended conformations; they facilitate direct mapping of unfolding/folding pathways and measurement of kinetics at the single-molecule level. By using force-measuring optical tweezers, we studied the mechanical unfolding and folding of a hairpin-type pseudoknot in human telomerase RNA in a near-physiological solution at room temperature. By stepwise force-drop experiments, discrete two-state folding transitions of the pseudoknot are seen at forces of $\sim$10 and $\sim$5 picoNewtons (pN), with molecular extension decreases of $\sim$10 and $\sim$7 nm, respectively, and ensemble rate constants of $\sim$0.1 s^{-1}. Folding studies of the isolated 5′ hairpin construct suggested that the 5′ hairpin within the pseudoknot forms first, followed by formation of the 3′ stem. Stepwise formation of the pseudoknot structure at low forces are in contrast with the one-step unfolding at high forces of $\sim$46 pN with molecular extension increases of $\sim$36 nm, at an average rate of $\sim$0.05 s^{-1}. In the folding experiments of constant-force at $\sim$10 and $\sim$5 pN, transient formation of non-native structures with similar molecular extensions as that of the native structure was observed, showing that folding of both the hairpin and pseudoknot takes complex pathways. Possible non-native structures and folding pathways are proposed.

1110-Pos Accurate Calculation of RNA loop Entropy

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The entropy is important for RNA stability, but it is difficult to measure or calculate. By analyzing the rotamers of RNA main chain, we built a *K*-state discrete model of RNA main chain. With this model and the sequential Monte Carlo (SMC) method, we calculated the entropy of RNA hairpin loops, bulges, internal loops, and multibranch loops. The calculated entropy of hairpin loop, bulge, and internal loops all agree well with experiments, suggesting that our model is exact. However, the calculated entropy of multibranch loops is much higher than experimental values for long chains, indicating the possibility that the extrapolation formula from experimental data needs to be revised. Our model has the advantages that

1. it can calculate the entropy of loop of arbitrary complexity.
2. it can calculate the loop entropy of very long chains (300~500 nucleotides) without resorting to extrapolations;
3. enabled by the accurate calculation, we can distinguish the entropy contribution from other factors, such as the noncanonical base pairs, coaxial stacking and etc;

we can also study the asymmetric effect systematically, which is very difficult in experiments, especially for multibranch loops. Our study will lead to better energy parameters for predicting RNA secondary structures and will be useful in RNA tertiary structure predictions.

1111-Pos Folding Of Small RNA Studied By SAXS

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Early steps in RNA folding determine the probability of forming the final compact structure that is essential for biological function. The *Azoarcus* ribozyme experiences three thermodynamic states: the unfolded RNA (**U**), a compact and native-like intermediate ($\mathbf{I_c}$), and native RNA (**N**). The first transition from **U** to $\mathbf{I_c}$ is ascribed to assembly of double helices in the center of the RNA that precedes the second transition from $\mathbf{I_c}$ to **N**. The $\mathbf{I_c}$ state is as compact as **N** state, suggesting that first transition is driven by native-tertiary interactions. To understand the microscopic mechanism of the folding process, we studied the time dependence of the folding transitions and the role of cooperative tertiary interactions in stabilizing the $\mathbf{I_c}$ and **N** states. The kinetics of ribozyme folding in the presence of Mg^{2+} was investigated by time-resolved small angle X-ray scattering (SAXS) using a stopped-flow sample chamber. The preliminary results indicate that the wild type *Azoarcus* ribozyme folds through multiple pathways, including fast folding within t ~ 10 ms and slow folding at t > 3 min. Most of the *Azoarcus* ribozyme (~ 85 %) participates in the fast-folding pathway, in agreement with the results of time-resolved hydroxyl radical foot-printing on the RNA backbone. Equilibrium Mg^{2+} titrations on the anti-sense RNA and mutants containing single-base substitutions demonstrate that perturbation of tertiary interactions leads to bigger R_g values than the fully folded structure, suggesting that tertiary interactions play important roles in driving the stabilization of the collapsed state.

1112-Pos Folding of the TPP riboswitch

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Thiamine pyrophosphate (TPP) riboswitches regulate the expression of thiamine-synthesis (*thi*) genes in organisms from all kingdoms of life(1) through a variety of mechanisms. Common to these mechanisms is binding of TPP to a structured aptamer in the untranslated region (UTR) of a *thi* mRNA. We used a high-resolution, single-molecule optical trapping assay to mechanically characterize folding of the aptamer for AtRs, the *Arabidopsis thaliana* TPP riboswitch, which is located in the 3′ UTR of the *thiC* gene. Each aptamer molecule was transcribed *in situ* by an RNA polymerase molecule, and placed into our "dumbbell" experimental geometry(2). By applying tension to the ends of the aptamer under both equilibrium and non-equilibrium conditions and measuring the molecular extension, we observed transitions among several intermediate folding states, which we discuss in the context of the secondary and tertiary structure of the aptamer(3). One state, corresponding to the presence of secondary structure at high force, is abolished by making a 1-nt mutation which is believed to disrupt the three-helix junction at the heart of the aptamer(3,4). The wild-type aptamer only binds to TPP while in the fully folded state, suggesting that aptamer folding obligatorily precedes TPP binding. We describe the energetics of folding and kinetics of TPP-binding for both the wild-type and mutant aptamers under various magnesium-ion concentrations, and present an energy landscape for the folding of the aptamer.

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1113-Pos Salt Dependence Of RNA Loop Stability And Hairpin Folding Free Energy

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Board B89

Single-stranded nucleic acid loops are important structural and functional segments. Due to the polyanionic nature of nucleic acids, metal ions in solutions are crucial to the loop folding thermodynamics. Here, we use the previously developed tightly bound ion theory [1,2] to quantify the contributions of Na^+ and Mg^{2+} to the loop thermodynamics, where the loop conformational ensembles are produced based on the virtual-bond model [3]. The predicted loop thermodynamics are strongly dependent on loop length, end-to-end distance, and Na^+/Mg^{2+} concentrations. Based on the calculations, we derive the empirical formulas for the loop thermodynamic parameters as functions of chain length, end-to-end distance, and Na^+/Mg^{2+} concentrations. For the three specific loops, namely, hairpin, bulge, and internal loops, the predicted entropies agree with the experimental data at standard salt (1M Na^+). Furthermore, we apply the calculated loop free energy to predict RNA hairpin stability in Na^+/Mg^{2+} solutions. The results show good agreement with available experimental data. We find that both helix and loop can cause significant salt-dependence for the hairpin stability.

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Virus Structure & Assembly

1114-Pos Tobacco Mosaic Virus Assembly of Fibrous and Macroscopic Bundled Arrays Mediated by Surface Aniline Polymerization

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One-dimensional (1D) polyaniline/tobacco mosaic virus (TMV) composite nanofibers were synthesized by the self-assembly of rod like TMV particles assisted by the polymerization of aniline on its surface. At near-neutral reaction pH (6.0 – 8.0), branched polyaniline formed on the surface of TMV prevented lateral association and therefore long 1D nanofibers were observed with high aspect ratios and excellent processibility. At a lower pH (4.0–5.0), transmission electron microscopy (TEM) analysis revealed that initially long nanofibers were formed and upon long reaction time resulted in bundled structures. This association of single nanofibers to form bundled structures is presumably mediated by the hydrophobic interaction of polyaniline on the surface of these composite nanofibers. In-situ time-resolved small-angle X-ray scattering study on the formation of polyaniline/TMV composite nanofibers at two different reaction pH conditions (5.1 and 6.5) supported our mechanism. The polyaniline layer formed on the external surface of TMV is thicker for reaction at pH 5.1 than for reaction at pH 6.5. This novel strategy to assemble TMV into well-organized composites could be utilized in the fabrication of advanced materials for potential applications including electronics, optics, sensing, and biomedical engineering.

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1115-Pos Plastic deformation of viral capsids

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From theoretical considerations on the response of viral capsids to deformation one expects that next to

(i) complete elastic deformation and
(ii) capsid failure upon deformation, there is a third possibility:
(iii) plastic deformation.

Whereas the first two regimes have already experimentally been observed (1, 2), the plastic deformation regime has not. Here we report on experiments in this intermediate response regime. The studies were performed with nanoindentation techniques using an Atomic Force Microscope (AFM), an approach which is becoming a standard method to measure the mechanical properties of viral particles (1, 2). Capsids of the Hepatitis B Virus (HBV) were used as a model system. This virus was chosen because its capsid is made up out of many copies of a single protein and it can form in either a T=3 or a T=4 morphology. After a certain indentation both capsid types show permanent plastic deformations in their structures and the mechanics of this deformation can be described by the Föppl-von Kármán (FvK) number. It is shown that the experimental observations match the expected behaviour as deduced from simulations (3). Overall the two morphologies possess the same mechanical characteristics, but a slight difference in deformation characteristics is predicted by theoretical considerations on the difference in FvK number. We are able to observe this difference experimentally, which illustrates the resolution of our approach.