antibiotics which target only a small number of bacterial processes. However, with the discovery of riboswitches, we are developing new ways to fight bacterial infections which make use of their own natural metabolic pathways, essentially causing bacteria to destroy themselves. Riboswitches are found in non-coding regions of messenger RNAs and these RNA elements bind to ligands to control the expression of nearby genes. The glucosamine-6-phosphate (glmS) riboswitch is unique in that upon binding its ligand, glucosamine-6-phosphate (GlcN6P), it undergoes self-cleavage and is therefore also a catalytic RNA. The cleavage event targets the RNA for subsequent degradation, thereby repressing further gene expression. To study the glmS riboswitch, initial experiments were performed to determine the mechanism followed upon binding of the natural ligand. Since then, analogs of the natural ligand have been obtained and are being tested for their catalytic capabilities through kinetic analyses and rate constant calculations. Once successful candidates have been determined, these non-natural ligands will be introduced into live bacterial cultures, hopefully disrupting normal cell metabolism and reproduction. If successful, these analogs could be used as novel antibiotics, offering a more specific mode of targeting a wide variety of bacterial species.

1371-Pos

Folding of the Thiamine Pyrophosphate (TPP) Riboswitch

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TPP riboswitches regulate the expression of thiamine-synthesis (*thi*) genes through a variety of mechanisms, all of which involve binding of TPP to a structured aptamer formed in the untranslated region (UTR) of a *thi* mRNA. We used a high-resolution, single-molecule optical trapping assay to characterize mechanically the folding of the TPP riboswitch aptamer located in the 3'UTR of the *thiC* gene of *Arabidopsis thaliana*. Each RNA molecule, containing either the complete aptamer sequence or a portion thereof, was transcribed *in vitro,* annealed to DNA handles via single-stranded overhangs, and placed in a ''dumbbell'' experimental geometry[1]. By applying tension to the ends of the RNA molecule under equilibrium conditions and measuring the corresponding extensions, we observed transitions among several well-defined folding states, which we discuss in the context of secondary and tertiary structures formed by the aptamer[2]. One low-force state of the full aptamer, corresponding to the formation of structural elements located near the three-helix junction, was abolished by mutating a single nucleotide believed to participate in specific tertiary contacts within the junction[2,3]. We observed that the mutant aptamer does not bind TPP or other substrates (thiamine, thiamine monophosphate), and that the wild-type aptamer only binds substrates concomitant with entry into the fully-folded state. We also studied the energetics of substrate binding under non-equilibrium conditions by rapidly increasing or decreasing the extension of the aptamer and measuring the hysteresis in force. The number of phosphates on the substrate modulated the amount of work required to induce substrate unbinding, the height and location of the energy barrier to substrate unbinding, and the amount of RNA released.

1. Greenleaf WJ, et al (2005). PRL 95, 208102.
2. Thore S, et al (2006). Science 312, 1208-1211.
3. Sudarsan N, et al (2005). Chemistry & Biology 12, 1325-1335.

1372-Pos

Structure and Function of a Potential Mammalian Riboswitch

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Riboswitches, found in untranslated regions of mRNAs, bind to specific cellular metabolites and undergo a conformational change which modifies expression of a nearby coding region of the mRNA. This coding region is involved in the synthesis of the same metabolite, thereby providing an efficient feedback mechanism of genetic control. To date, various riboswitches have been described to effectively control genetic expression in bacterial cells, but none have been discovered in mammals. We are investigating the structure and function of a potential mammalian riboswitch, thought to control polyamine biosynthesis. Polyamines surround cellular DNA to stabilize the DNA negative charge. To validate this small RNA as a new riboswitch, we are using in-line probing to verify specific metabolite binding and subsequent conformational change. Additionally, to verify the ability of the potential riboswitch to control gene expression, in vivo studies are being performed using a reporter gene system. Successful results from both of these investigations will determine whether this small RNA is a true riboswitch. Further investigations will include determination of its tertiary structure. It is known that cancer cells require a higher concentration of polyamine due to their increased replication rate. Thus, a combination of structural and functional studies of this RNA may prove useful in the development of novel cancer therapies.

1373-Pos

Dynamics of the Catalytic Pocket of a Diels-Alder Ribozyme

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The Diels-Alder ribozyme, an in vitro-evolved highly abundant ribonucleic acid enzyme, accelerates the formation of carbon-carbon bonds between a diene and a dienophile in a [4+2] cycloaddition reaction, a reaction with broad application in biochemistry and organic chemistry.

We have examined the ribozyme in the unbound form in solid and liquid phase by means of Molecular Dynamics simulations of 1 microsecond total simulation time. Our simulations confirm highly dynamic state of the catalytic pocket as observed by recent NMR spectroscopy studies.

However, the preformed catalytic pocket architecture, suggested previously based on X-ray investigations, exists only under certain conditions. Simulations of the crystal state show that at the temperature of 100K the catalytic pocket remains in its starting conformation. Yet, at the transitional temperature of 250K a collapse of the catalytic pocket occurs, and the ribozyme adopts an ezymatically inactive closed conformation of the pocket.

Simulations in solution performed at 300K at different magnesium ions concentration reveal that the stabilization of the catalytic pocket depends on high amounts of Mg-ions. At higher Mg^{2+} concentrations the cations are more likely to bind to the backbone of those residues that bridge the opposite strands of the pocket, which leads to stabilization of the enzymatically active open conformation. Simulations with artificial constraints confirm and quantify the effect of backbone stabilization on a catalytically active state. At too low Mg-ion concentrations, catalytically inactive states with a collapsed catalytic pocket dominate. In these conformations the ribozyme is not able to host any reactant. The catalytically active state with an open pocket is a metastable state that can only be accessed and is only sufficiently stabilized at a high enough magnesium concentration, explaining the experimentally found full catalytic activity dependence on the Mg-ions concentration.

1374-Pos

Structural Probing of the T Box Antiterminator-tRNA Complex

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Structural changes in a unique RNA-RNA binding interaction were probed using 2-aminopurine. The 5′-untranslated leader region (5′-UTR) of the T box family of genes folds into a structure that selectively recognizes a specific tRNA through two unique base-pairing events. The first involves base pairing between the anticodon of cognate tRNA and a tri-nucleotide sequence (specifier sequence) in the specifier loop of stem 1 in the 5′-UTR. The second base pairing event involves the non-aminoacylated tRNA acceptor end base pairing with the first four nucleotides at the 5′-end of a bulge in a highly conserved antiterminator element. In the absence of the stabilization of the uncharged tRNA acceptor end base pairing to the antiterminator element, the more thermodynamically stable terminator element forms and transcription terminates. In this manner, the leader region specifically recognizes cognate tRNA and responds structurally to the charging ratio of the tRNA to regulate transcription, thus making the T box mechanism an example of a riboswitch. Interestingly, the predicted thermodynamic stabilization provided by the four base pairs between the tRNA acceptor end and the antiterminator is not sufficient to overcome the predicted stability difference between the antiterminator and the terminator elements. Consequently, additional structural factors likely play a role in stabilizing the resulting complex. The structural changes induced in both the antiterminator element and the tRNA were investigated using a model system to determine what additional factors, beyond base pairing, contribute to stabilization of the resulting tRNA-antiterminator complex. Fluorescence monitoring of the base analog 2-aminopurine at select positions throughout a model complex indicated that binding results in an induced-fit and a highly stacked environment at the binding interface. These structural features contribute to the overall stabilization of the complex beyond the four base pairs.

1375-Pos

Nanosecond Motions of the Substrate-Recognition Duplex in a Group I Intron Assessed by Site-Directed Spin Labeling

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The *Tetrahymena* group I intron recognizes its oligonucleotide substrate in a two-step process. First, a substrate recognition duplex, called the P1 duplex,

is formed. The P1 duplex then docks into the pre-folded ribozyme core by forming tertiary contacts. P1 docking controls both the rate and the fidelity of substrate cleavage and has been extensively studied as a model for the formation of RNA tertiary structure. However, previous work has been limited to studying millisecond or slower motions. Here we investigated nanosecond P1 motions in the context of the ribozyme using site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) spectroscopy. A nitroxide spin label was covalently attached to a specific site of the substrate oligonucleotide, the labeled substrate was bound to a pre-folded ribozyme to form the P1 duplex, and X-band EPR spectroscopy was used to monitor nitroxide motions in the 0.1 to 50 ns regime. Using substrates that favor the docked or the undocked states, it was established that the nitroxide was capable of reporting P1 duplex motions. Using these nitroxide labeled substrates, it was found that the J1/2 junction connecting P1 to the ribozyme core controls nanosecond P1 mobility in the undocked state (Grant et.al., 2009, JACS, 131, 3136-7). This may account for previous observations that J1/2 mutations weaken substrate binding and give rise to cryptic cleavage. This study establishes the use of SDSL to probe nanosecond dynamic behaviors of individual helices within large RNA and RNA/protein complexes. Work is underway to investigate P1 motions in various mutant ribozymes in order to establish detailed correlations between nanosecond dynamics of P1 with ribozyme tertiary folding and catalytic activity. This may help in understanding the relationship between RNA structure, dynamics, and function.

1376-Pos

Mapping the Global Conformation of the Phi29 Packaging RNA Dimer Using Deer Distance Constraints

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The insertion of bacteriophage phi29 genomic DNA into its preformed procapsid requires the DNA packaging motor, which is the strongest known biological motor. The packaging motor is an intricate ring-shape protein/RNA complex. The RNA component, called the packaging RNA (pRNA), is indispensable for motor function, and may play an essential role in motor ATPase activity. Current structural information on pRNA is limited, which hinders our effort on understanding motor function. Here, we use site-directed spin labeling and pulse EPR spectroscopy to map the global structure of a pRNA dimer that has been shown to be a functional intermediate in assembling the ring-shaped pRNA complex in the packaging motor. In our studies, nitroxide pairs were attached to specific sites of a truncated monomeric pRNA construct, the labeled monomers were then assembled into dimers in the presence of Mg2+, and inter-nitroxide distances were measured using DEER (Double Electron-Electron Resonance) spectroscopy. In parallel, an unbiased pool of models that contains variable pRNA conformations was generated, which treats pRNA as a 3-way junction construct, and a set of corresponding inter-nitroxide distances was predicted for each model. Intra-molecular DEER distances were used to obtain the monomeric structures of pRNA in dimer, which are then used to build the structural pool for pRNA dimers. A very small number of models were selected. We expect that this work will provide much-needed structural information regarding pRNA, as well as establishing a new methodology for analyzing global conformations in complex RNAs.

1377-Pos

Accurate Distance Constraints for RNA Structures Using Deer Spectroscopy

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Understanding structure-function relationships in RNA and RNA-protein complexes requires robust methods for obtaining structural information on a variety of length scales. DEER (double electron-electron resonance) is emerging as a powerful method for very accurate ($\pm$ 2 Å over 15-80 Å) distance measurements between pairs of nitroxide labels that can be placed using several available conjugation sites in RNA nucleobases or phosphodiester linkages. Here, we show the potential for DEER spectroscopy in monitoring global RNA folding and also small changes in RNA structure within a model system that is based on the Hammerhead ribozyme. This catalytically active RNA, a three-helix junction motif with a buried active site, undergoes cation-dependent folding transitions that are linked to activity. Nitroxide labels placed at strategic positions allow helix-docking and active-site core rearrangements to be monitored by measuring the dipolar coupling between paramagnetic sites. This poster will present the results of DEER measurements obtained at both X-band and Q-band, where the higher-frequency Q-band spectroscopy significantly enhances the sensitivity of this technique. Mg^{2+}-dependent global folding, and evidence for a smaller local structural change with higher added metal concentrations, are both observed in this RNA. Since labels can be placed at targeted sites within both nucleic acids and proteins, and there is no inherent limitation on macromolecular size, DEER spectroscopy has potential for obtaining high-resolution structural information in complex RNAs and in large RNA or DNA-protein complexes.

1378-Pos

Filtering RNA Decoys with Small Angle X-Ray Scattering and Clustering Analysis

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RNA molecules have previously been regarded as ''boring'' molecules which merely relay genetic information from DNA to proteins. However, they are now known to also exhibit a wide range of gene regulation functions. For instance, the RNA riboswitch binds to ligands, and then undergoes structural changes that regulates either transcription or translation. Like proteins, the function of an RNA depends on its three dimensional structure. Here, we discuss how we can incorporate low-resolution experimental data (namely, small angle x-ray scattering) to score the RNA models (also known as decoys). We have also studied the similarities of clusters from multiple k-means clustering runs on the decoys to help us distinguish well-sampled predicted structures from noise. Use of this clustering analysis allows us to effectively reduce the effects of outliers, which commonly plague low-resolution experimental scoring functions.

1379-Pos

Molecular Dynamics and Distribution of Ions in Kissing Loop

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RNAs have hierarchical folding of structure which is endowed with abilities to catalyze biochemical reactions, support ligand binding, and proteins recognition. Ionic environment assist RNA to form stable higher order structures. In this study, molecular dynamics simulations were used to analyze the monovalent cationic distributions within RNA loop-loop complexes taken from separate viral species. We demonstrate that cations in show strong preferential distribution around kissing loop region however, ion dynamics do not indicate concrete evidence of specific binding. Cationic spatial localization was observed in a variety of kissing loops. Simulations results reveal the presence of electronegative channels that formed through the major groove of all RNA loop-loop helices and attract and retain the cations. Significant drop of diffusion coefficients was observed for ions inside ionic channels. Effect of sequence on the ion distribution was observed by carrying out mutational studies on the bacterial and viral kissing loops. Molecular dynamics results show strong correlation of ionic propensity regulated by sequence.

1380-Pos

Portability of a Common Nucleic Acid Hairpin Loop Motif Between RNA and DNA

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Hairpins are common nucleic acid secondary structures that perform both structural and functional roles. Recently, we reported that r(UNCG) and r(GNRA) hairpin families use molecular mimicry and electrostatic factors to attain exceptional thermodynamic stability with a CG closing base pair (cbp) (*J. Amer. Soc.* **2009** 131, 8474-8484). Although these loop families fold with different global structures, the tetraloops are stabilized by displaying the same functional groups and partial charges to the major groove edge of the CG cbp. Herein, we compare the r(GNRA) tetraloop family to the DNA triloop family d(GNA), which is also exceptionally stable with a CG cbp and possesses same sheared GA base pair between the first and last positions of the loop (*Biochemistry* **2009** 48, 8787-8794). Interactions of d(GNA) loops with the cbp were probed with nucleobase and functional group modifications and the resulting effects on stability were compared to those from similar substitutions in r(GNRA) hairpins. Interruption or deletion of loop-cbp interactions in d(GNA) was consistent with electrostatic interactions identified through nonlinear Poisson-Boltzmann (NLPB) calculations. Moreover, loop stability changed in a manner consistent with similar loop-cbp interactions for d(GNA) and r(GNRA) loops. We also compared the relationship of ΔG°_{37} and log[Na^{+}] for d(GNA) and r(GNRA) loops and found a decreased salt dependence for both loop families with a CG cbp. Similarity of loop-cbp interactions shows portability of the loop-cbp motif across polymer type and loop size and indicates RNA and DNA converged on a similar molecular solution for stability.