in between, with the upper helix capped by GAAA tetraloop. The NMR titration of magnesium ions with D5 indicates that it act as a potential metal binding platform. Here we are reporting the structure of a deletion mutant of D5 which is defective in catalysis of the substrate, but effective in binding to D123 domain.

1297-Pos Board B207
The Multiple Substrate Recognition Properties of Ribonuclease P: Achieving Uniformity in Processing Kinetics Despite Variation in Substrate Structure
Lindsay E. Yandek, Michael E. Harris.
Ribonuclease P is an essential enzyme that is responsible for catalyzing the maturation of the 5' end of transfer RNA. In Bacteria, the RNase P holoenzyme is composed of a RNA subunit and a protein subunit. The RNA subunit of RNase P contains the enzyme active site and has the ability to process substrates in the absence of the protein component *in vitro*, but works as a holoenzyme *in vivo*. While nucleotide recognition elements adjacent to the site of processing have been identified, different tRNA sequences vary considerably. Remarkably, rates of pre-tRNA processing are uniform despite this variation in substrate sequence and structure. The mechanistic basis for multiple substrate recognition by the holoenzyme is the focus of this study, with the ultimate goal being a better understanding of uniformity in ptRNA processing and discriminates between cognate and non-cognate RNAs. We are determining kinetic schemes for a number of pre-tRNAs using fluorescence assays and standard discontinuous assays. Multiple turnover kinetics have been obtained for ptRNA608^{MET}, a consensus pre-tRNA, and ptRNA605$^{f\text{-}MET}$ a non-consensus sequence. Both ptRNAs display a similar V_{max}, and Km values. Neither tRNA displayed any burst nor lag phases in pre-steady state kinetics implying that the rate limiting step for our processing model is catalysis. Our initial hypothesis is that uniformity in substrate kcat/Km values results from differential 5' leader sequence interactions with the protein that compensate for deviations from tRNA consensus recognition sequences. To obtain more insight into sequence and structure influence, we have set up a series of multiple turnover experiments with a consensus and non-consensus ptRNA competing against one another.

1298-Pos Board B208
Measuring the Dimensions of a Compact Kinetic Intermediate in the Folding Pathway of the GlmS Ribozyme
Steve Meisburger, Krista Brooks, Suzette Pabit, Li Li, Joshua Blose, Ken Hampel, Lois Pollack.
Using complementary time-resolved biochemical and x-ray probes of RNA structure in solution, we investigate the cation-induced folding of the glmS ribozyme, a metabolite-sensing RNA switch that regulates gene expression in bacteria. Hydroxyl radical footprinting experiments have shown a concerted folding transition within the first 10 seconds after adding magnesium. From small angle x-ray scattering (SAXS) experiments performed under similar conditions, we find that native tertiary contact formation is preceded by the collapse of the molecule to a relatively compact intermediate. The subsequent compaction observed by SAXS correlates temporally with changes in hydroxyl radical protection. We propose a structural model for the intermediate and possible implications for the role of secondary structure and electrostatics in the folding process of this ribozyme.

1299-Pos Board B209
Folding Kinetics for the Conformational Switch Between Alternative RNA Structures
Song Cao, Boris Furtig, Harald Schwalbe, Shi-Jie Chen.
The conformational switching between different conformational states is intrinsic to RNA catalytic and regulatory functions, which oftenly occurs on time-scales of several seconds. In combination with the recent real-time NMR experiments (Wenter et al. Angew. Chem. Int. Ed. (2005). 44, 2600; Wenter et al. ChemBioChem. (2006). 7, 417) for the transitions between bistable RNA conformations, we combine the master equation method with the kinetic cluster method to investigate the detailed kinetic mechanism and the factors that govern the folding kinetics. Based on the computational studies, we propose that heat capacity change upon RNA folding may be important for RNA folding kinetics. In addition, we find that noncanonical (tertiary) intraloop interactions in tetraloop hairpins are important to determine the folding kinetics. Furthermore, through theory-experiment comparisons, we find that the different rate models for the fundamental steps (i.e., formation/disruption of a base pair or stack) can cause contrasting results in the theoretical predictions.

1300-Pos Board B210
Structure and Dynamics of "SWITCHING" RNAs
T. Kwaku Dayie.
To date, most RNA structural information have been derived almost entirely from either X-ray crystallography or NMR spectroscopy. Even though X-ray crystallography has no size limit, there are concerns about potential crystal packing artifacts and a large number of RNAs remain refractory to crystallization. NMR is uniquely placed to tackle those classes of macromolecules that resist crystallization. To play an effective role, however, at least two inherent limitations of NMR that include signal overlap and rapid signal decay need to be circumvented. In the past, uniform isotopically enriched RNA samples have been used to determine the structures of small (<30 nucleotides) RNAs by NMR. However, as RNAs become larger and more interesting, the proton and carbon spectra of uniformly labeled RNA samples become a tangled web of overlapping peaks, severely limiting the usefulness of NMR. As an effective workaround, we have developed an array of new selective labeling schemes based on biomass nucleotide production using various bacterial strains, and we have designed new NMR experiments to exploit these labels. To test the hypothesis that some riboswitches employ conformational structural rearrangements to sequester the Shine-Dalgarno sequence in response to metabolite binding, we have initiated NMR studies of ligand-free riboswitches using our selective labels. We show that in the absence of ligands, there is a small population of G-C Watson-Crick base pairs, suggesting that the ligand-free riboswitch, though partially folded, samples some of the folded conformation in solution. Our methodology should be broadly applicable to other RNAs that require "switching" for their function.

1301-Pos Board B211
Ligand Induced Conformational Changes of Riboswitches Probed by SAXS and NMR Spectroscopy
Bin Chen, Xiaobing Zuo, Yunxing Wang, T. Kwaku Dayie.
Ribsowitches are a newly discovered large family of structured RNA elements that are typically located at 5' untranslated regions of messenger RNAs. They are composed of two domains: aptamer domain and expression platform. The aptamer domain functions as a sensor to recognize and bind specifically cellular metabolites while expression platform modulates gene expression in response to the conformational changes in the aptamer by either inhibiting transcription elongation or blocking translation. More than twenty riboswitch subfamilies have been identified so far in a variety of organisms ranging from bacteria, fungi to plants, indicating that riboswitch is a widespread genetic control element. Structural studies of ligand-bound riboswitches by X-ray crystallography and NMR spectroscopy provide insight into detailed RNA/ligand recognition and interactions. The structure, however, of ligand-free riboswitches remains poorly characterized. For better understanding the mechanism of riboswitches' functions and their transition from ligand-free and ligand-bound forms, it is critical to characterize the molecular details of the unliganded state. We have employed a variety of biochemical, biophysical, and computational techniques including SAXS and NMR spectroscopy to characterize the ligand-free and ligand-bound forms of riboswitches. Our data reveal that ligand binding of the RNAs causes significant conformational change and that only after ligand-binding does the formation of various secondary and tertiary structural elements occur.

1302-Pos Board B212
Exploring Wild-Type and Mutant E. coli Strains for the Synthesis of Site-Specific Labels to Study RNA Structure and Dynamics by NMR
Jacob N. Sama.
Ribonucleic acids are involved in many biological processes including catalysis, transfer and translation of genetic material, and regulation of gene expression. This unique ability to perform a variety of functions, traditionally associated with proteins is largely due to their capacity to adopt three-dimensional structural folds. Studying the 3D architecture of RNAs is critical for not only understanding the molecular basis of RNA function, but will eventually help with structure-assisted drug design, discovery and delivery. Heteronuclear NMR has become a powerful tool for studying the structure and dynamics of RNAs. To date, several RNAs have been well characterized by this method. However, overcrowding of chemical shifts and rapid signal loss in larger RNAs renders current NMR methods ineffectual. To study the structure and dynamics of larger RNAs, the use of site-selectively 13C-labeled nucleotides promises to be very helpful. To synthesize these labels, it was hypothesized that the metabolic pathways of various Escherichia coli wild type and mutant strains are capable of producing specifically labeled nucleotides necessary for making RNA. To test this hypothesis, we evaluated the growth of mutant strains K10-1516 (deficient in glucose-6-phosphate dehydrogenase of the pentose phosphate pathway) and DL323 (deficient in the Krebs

cycle) on optimal LeMaster Richards's minimal media supplemented with carbon source 2-13C-glycerol alone or in combination with 13C-formate to enhance labeling at carbon positions bearing protons that are useful for NMR studies. Experimental results showed that the strains produced specific ribose and nucleotide labels that were readily predicted. These labels will enable us to study the structure, function and dynamics of higher size RNA molecules using NMR that would have been otherwise difficult if the commercially available uniformly labeled or unlabeled ribonucleotides were utilized.

1303-Pos Board B213

Characterization of ps-Ms Dynamics in the TAR Apical Loop by NMR

Elizabeth A. Dethoff, Hashim M. Al-Hashimi.

The transactivation response element (TAR) is located at the 5′ end of the HIV-1 genome and regulates the transcription elongation step of viral RNA. The TAR stem-loop binds the HIV viral transactivator protein (Tat) and human positive transcription elongation factor b (P-TEFb), leading to productive transcription of the HIV genome. The formation of the TAR/Tat/P-TEFb ribonucleoprotein complex remains poorly understood from a structural and dynamical standpoint. To better understand its formation, we have studied the structural and dynamic features of free TAR to elucidate motions in both the bulge and apical loop that may be important for adaptive recognition of protein targets.

A combination of nuclear magnetic resonance (NMR) relaxation techniques, including ^{13}C relaxation (R_1, R_2) and ^{13}C $R_{1\rho}$ relaxation dispersion, were used to characterize local and global dynamics at the ps-ns timescale and to site-specifically quantify slow motions on the us-ms timescale, respectively. ^{13}C $R_{1\rho}$ relaxation dispersion reveals the presence of us-ms exchange in the loop caused by the existence of ''invisible'' excited states. Full characterization of these excited states may give insight into the recognition of Tat and P-TEFb by TAR. In general, our results reveal that the apical loop and bulge undergo complex dynamics at multiple timescales that are likely important for adaptive recognition.

1304-Pos Board B214

Site-Pecific Fluorescnce Dynamics in an RNA 'THERMOMETER' Reveals the Mechanism of Temperature-Sensitive Translation

Mamata Kombrabail, Suman Paul, Basukthar J. Rao,
Guruswamy Krishnamoorthy.

The ROSE (**R**epression **O**f heat **S**hock gene **E**xpression) element of mRNA present in the 5'-UTR of small heat-shock genes in many Gram-negative bacteria is known to function as a 'RNA thermometer' by controlling protein translation in a temperature range of 30 - 42° C where the translation is blocked till 30°C and allowed at 42°C and beyond, perhaps due to an unfolding transition of the ROSE hair-pin motif.

In this work, we have used site-specific fluorescence labeling and pico-second time-domain fluorescence spectroscopy to unravel the mechanism. The 'ROSE RNA' was site-specially labeled with 2-aminopurine (2-AP), a fluorescent analog of adenine. Observables such as fluorescence lifetime, fluorescence anisotropy decay kinetics and dynamic fluorescence quenching revealed properties such as the level of base stacking, rotational motion of the bases, segmental dynamics of the backbone and the level of exposure of base to solvent. As expected, all read-outs of 2-AP residue that were studied showed remarkable position-dependence/sensitivity in the RNA sequence at 25°C. The striking result was the persistence of the same position-dependence of the parameters even at 45°C albeit at a measurably reduced levels. However the same position-dependence was nearly 'wiped out' in the presence of urea where all intra-molecular interactions in RNA are undone. These observations have prompted us to revise the existing model of ROSE RNA action: we now suggest that unlike proposed earlier, the thermometer action of ROSE emanates not from its unfolding structural transition between 25 and 45°C, but rather from its propensity to enhance structural dynamics without ''melting'' the structure. We hypothesize that either the enhanced dynamics of the structure it self or its full melting due to an extrinsic factor (perhaps a protein interaction) might be the basis of its thermometer action.

1305-Pos Board B215

Revealing the Energy Landscapes of Ribosome Function

Paul C. Whitford, Christian M.T. Spahn, Scott C. Blanchard,
Jose' N. Onuchic, Karissa Y. Sanbonmatsu.

The ribosome is a massive ribonucleoprotein complex (~2.4 MDa) that harnesses large-scale structural fluctuations to produce unidirectional protein synthesis. We address the relationship between ribosome energetics, structural fluctuations and biological function via all-atom molecular dynamics simulations. Specifically, we utilized large-scale explicit-solvent simulations (3.2 million atoms), in addition to models that employ simplified energetics (~150,000 atoms) to describe the microsecond to millisecond processes associated with transfer RNA molecules as they enter, and move through, the ribosome. By simulating ribosomal hybrid-state formation, we have identified common physical principles that guide multiple rearrangements during ribosome function. This work demonstrates that the configurational entropy contributes significantly to the landscape, which has implications for fidelity and efficiency of ribosome function.

1306-Pos Board B216

A Coarse Grain RNA Model for Exploration of RNA Conformational Space

Anthony M. Mustoe, Hashim M. Al-Hashimi, Charles L. Brooks III.

Several recent studies have suggested that RNA three-dimensional structure and dynamics are highly restricted to a small set of allowed conformations by topological constraints that are encoded at the secondary structure level. We have developed a coarse-grained model of RNA implemented within the CHARMM molecular dynamics package that allows us to further characterize the nature of RNA topological constraints. In this coarse grain model, each residue is represented using three pseudo-atoms for the phosphate, sugar, and base moieties respectively. Secondary structure is specified by modeling bonds between paired bases and parameterizing these regions to adopt A-form helical structure. All non-base paired residues are modeled without torsional potentials or attractive non-bonded forces, preserving only connectivity and repulsive steric terms. Thus, the energy landscape between different helical orientations is effectively flat, allowing efficient exploration of topologically allowed conformations.

We benchmark our simulations using results from prior NMR and bioinformatics studies of two-way helix junctions. Moreover, simulations starting from a linear chain of the 76 residue tRNA-Phe molecule show that our model is able to sample the native conformation with minimal computational effort. We also show that the size of the conformational ensemble is reduced by over an order of magnitude when a limited set of three non-crystallographically determined tertiary contacts are used as restraints. In fact, the mean all phosphate RMSD over an ensemble of 100,000 structures has a value of 10 Å. We also present preliminary results of simulations done on RNAs with greater than 200 residues. These results suggest topological constraints alone, coupled with a few important tertiary contacts for larger RNAs, are enough to significantly constrain the available conformational ensemble and suggest a new approach to RNA structure prediction that is applicable to very large RNAs.

1307-Pos Board B217

Computing the Conformational Entropy for RNA Folds

Liang Liu, Shi-jie Chen.

We develop a polymer physics-based method to compute the conformational entropy for RNA tertiary folds, namely, conformations consisting of multiple helices connected through cross-linked loops. The theory is based on a virtual bond conformational model for the nucleotide chain. A key issue in the calculation of the entropy is how to treat the excluded volume interactions. The weak excluded volume interference between the different loops leads to the decomposition of the whole structure into a number of three-body building blocks, each consisting of a loop and two helices connected to the two ends of the loop. The simple construct of the three-body system allows an accurate computation for the conformational entropy for each building block. The assembly of the building blocks gives the entropy of the whole structure. This approach enables treatment of molten globule-like folds partially unfolded tertiary structures for RNAs. Extensive tests against experiments and exact computer enumerations indicate that the method can give accurate results for the entropy. The method developed here provides a solid first step toward a systematic development of a theory for the entropy and free energy landscape for complex tertiary folds for RNAs and proteins.(Liu,L. and Chen,S.-J., J. Chem. Phys, 132, 235104; doi:10.1063/1.3447385).

1308-Pos Board B218

Fibonacci Primes and Topological Biomolecular Mechanics

Okan Gurel, Demet Gurel.

Leonardo Fibonacci (c.1170-c.1250) in his book *Liber Abaci* (1202) presented two sequences: Fibonacci (Arithmetic) Sequence, Fa, ([1], p.260), and Fibonacci (Geometric) Sequence, Fg, ([1], p.404). We show that when Fg {Congruence (mod Fg6)} prime factorized, reveals 11 primes, which we named