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

Fibonacci Primes identifying Fibonacci Primes Hexagon and Fibonacci Primes Spider. The center of the spider consists of Fibonacci primes 17 and 19 which move in the direction of increasing Fg numbers as congruence (mod 3), and the spider enlarges in a systematic pattern. Fa and Fg sequences determine specific positions in applications of Fibonacci primes to the two asymmetric halves of tRNA, mRNA, as well as protein biomolecules. [1] L.E. Sigler, *Fibonacci's Liber Abaci: A Translation in Modern English of Leonardo Pisano's Book of Calculations*, New York, Springer-Verlag, 2002.

## DNA Replication, Recombination & Repair

**1309-Pos Board B219**

**Single-Molecule Studies of the Eukaryotic Replicative DNA Helicase MCM2-7**

**Hasan Yardimci**, Antoine M. van Oijen, Johannes C. Walter.

In eukaryotes, double hexamers of the replicative DNA helicase MCM2-7 are loaded onto double-stranded DNA (dsDNA) at each origin of replication in the G1 phase of the cell cycle. In S phase, numerous accessory factors activate the helicase activity of MCM2-7, leading to unwinding of the DNA template. Despite decades of study, it remains unclear how MCM2-7 unwinds DNA. One model is that upon activation, MCM2-7 goes through a conformational change to encircle single-stranded DNA (ssDNA) whereupon it translocates along one strand while excluding the other (steric exclusion). An alternative model envisions that MCM2-7 translocates along dsDNA. As DNA emerges the rear exit channel of MCM2-7, it is split by a rigid pin that bisects the channel. To distinguish between the two scenarios, we used an experimental system that allows single-molecule visualization of DNA replication in *Xenopus* egg extracts. We attached a quantum-dot (Qdot) to lambda DNA at a specific location that served as a road block for replication forks. Upon exposure of such Qdot-labeled lambda DNA to extracts in a microfluidic flow cell, a significant fraction of forks bypassed the Qdot when it was located on the lagging strand template but not when it was located on the leading strand template. Our results support the model that MCM2-7 translocates in the 3' to 5' direction along ssDNA and unwinds DNA by sterically excluding the opposite strand. Our results suggest that the large number of factors that are required to activate the MCM2-7 complex at the G1/S transition function by remodeling the MCM2-7 complex from a dsDNA binding mode to a ssDNA binding mode.

**1310-Pos Board B220**

**Dna Unwinding Dynamics of a Processive DNA Polymerase**

**Borja Ibarra**, Jose Morin, Francisco Cao, Margarita Salas,
Jose M. Valpuesta, Jose L. Carrascosa.

During DNA replication mechanical unwinding of the DNA helix is required for the advance of the replication machinery. Unlike many DNA polymerases the bacteriophage Phi29 DNA polymerase presents a processive 'helicase-like' activity and is able to couple DNA replication and unwinding within the same polypeptide. Using Optical Tweezers we have developed a single molecule mechanical assay to elucidate the physical mechanism of DNA unwinding by the Phi29 DNA polymerase as the protein replicates processively the DNA. A DNA hairpin is hold between an optical trap and a mobile surface. As a single polymerase works on the hairpin its replication and unwinding activities can be measured in real time (by measuring the change in extension in the DNA polymer), revealing the fluctuations of their rates in response to the DNA sequence and force applied in the direction of unwinding. The sequence and force sensitivities of the unwinding reaction of the wild type and an unwinding-deficient polymerase mutant indicate that the Phi29 DNA polymerase presents an active unwinding mechanism that may substantially differ from the unwinding mechanism used by specialized nucleic acid helicases.

**1311-Pos Board B221**

**Recombination Hotspots and SSB Proteins Couple Translocation and Unwinding Activities of the AddAb Helicase-Nuclease**

Joseph T.P. Yeeles, Kara van Aelst, Mark S. Dillingham,
**Fernando Moreno-Herrero**.

Recombinational repair of DNA breaks requires processing of a DNA end to a 3′-ssDNA overhang. In B.subtilis, this task is done by the helicase-nuclease AddAB which generates ssDNA overhangs terminated at a recombination hotspot (Chi) sequence. In this work, we have used stopped flow DNA unwinding assays and atomic force microscopy to investigate the processing of DNA breaks by the AddAB helicase-nuclease. In the absence of single-stranded binding proteins, we found that translocation and unwinding activities of AddAB are uncoupled due to re-annealing of nascent single-stranded DNA. However, recognition of Chi sequences during AddAB translocation activates unwinding by coupling both activities. Helicase activity of AddAB is also activated by binding of SSB proteins or activity of multiple AddAB in multiple turnover reactions by preventing re-annealing of DNA strands. The implications of these findings for our understanding of DNA break repair intermediates and of general helicase mechanisms will be discussed.

**1312-Pos Board B222**

**Segregation of Sister Chromosomes in E. coli is Governed by the Shape of the Nucleoid and the Release of Inter-Sister "snaps"**

**Jay K. Fisher**, Aude Bourniquel, Mara Prentiss, Nancy Kleckner.

E. coli chromosome dynamics occurring throughout the cell cycle, as defined by fluorescence microscopy of living cells grown in a microfluidic device, have been analyzed in 3D at high resolution. Imaging of the nucleoid (HU-mCherry) reveals an asymmetric left-handed helicoidal ellipsoid. The shape can be seen to exert radial pushing forces at points of contact with the cell periphery, thereby defining complementary intracellular compartments. These compartments constrain the location of DNA replication and determine the paths taken by newly replicated DNA. As replication proceeds, two types of abrupt changes are obsserved: (i) rotation and modulation of the helix without change in basic shape; and (ii) longitudinal protrusion, first of one sister nucleoid towards the old cell pole and then of the other sister nucleoid towards the new cell pole. These two protrusion steps correspond to previously-described transitions involving release of specialized inter-sister ''snaps''. We propose that loss of the inter-sister ''snaps'' and ensuing nucleoid movements, as well as other dynamic effects seen later in the cell cycle, are driven by accumulation and release of mechanical stress arising from the intra-nucleoid repulsive forces, likely arising via interactions between negatively supercoiled plectonemes. We further propose that nucleoid shape arises via repulsion under confinement. Overall these findings lead to a picture in which the E.coli nucleoid is a ''bag of springs'', not a ''bag of string'', and where intrinsic physical properties of the DNA dictate the behaviors and dispositions of other components via mechanically-driven effects.

**1313-Pos Board B223**

**Mechanism of Yeast Clamp Loading on DNA**

**Tae-Hee Lee**, Stephen Benkovic, Padmaja Mishra, Ravindra Kumar.

Based on biochemical and single molecule fluorescence measurements using the yeast proliferating cell antigen (PCNA) and replication factor C (RFC), we studied the assembly of the RFC·PCNA·DNA complex and its progression to holoenzyme in the presence of polymerase δ (Pol δ). Our data indicate that i) PCNA loads to DNA through multiple conformational states; ii) PCNA loading is successful after several failed attempts; iii) there are two different states of PCNA loaded on DNA; iv) in the presence of Pol δ only one of the two states proceeds to the RFC·PCNA·DNA·Polδ holoenzyme. These findings redefine and deepen our understanding of the clamp loading process and reveal that it is surprisingly one of trial and error to arrive at a heuristic solution.

**1314-Pos Board B224**

**Nucleotide and DNA-Induced Structural Transitions and the Coupling Between ATP and DNA Binding Sites in RecA**

**Tao Jiang**.

RecA is the prototype of ATPase proteins that mediates homologous DNA recombination. RecA requires ATP to promote the binding of DNA, as both ADP- and nucleotide-free states show low DNA binding affinity. We investigated how ATP binding affects the dynamics of DNA binding Loops, and activates RecA by the interactions between β-/γ-phosphate and Walker A and Walker B motifs, as well as through the contacts made between the C-terminal and the central domains within the protein. DNA binding results in the formation of the extended, active conformation of the RecA filament, which catalyzes strand exchange. We have performed a set of molecular dynamics simulations on the active RecA, with ATP/ADP/nucleotide-free bound, to investigate the conformational transitions between the active and inactive states. Our simulations have revealed that the structural changes upon ATP binding are confined to small motifs, while the conformational changes upon DNA binding involve larger scale rearrangement of the protein, namely the rotation of monomers with respect to each other. The results suggest that ATP binding stabilizes the L1 and L2 DNA binding loops, mediated through specific residues located between the ATP and DNA binding sites that sense the presence of γ-phosphate. Furthermore, DNA binding leads to monomer rotations to form the extended conformation by affecting the interfaces of the adjacent monomers with