of BipA using amide hydrogen/deuterium exchange mass spectrometry (HDXMS). These data indicate that GTP- and ppGpp-binding lead to large scale conformational changes that are propagated throughout BipA, underscoring the idea that BipA is a metastable molecule where mutually exclusive association of GTP or ppGpp drive equilibrium to alternate distinct conformations resulting differential ribosome binding. Molecular dynamics simulations together with covariance analysis are being used to explore the dynamic allosterity between the GTPase and novel C-terminal domain.

1326-Pos Board B218
Single-Molecule Fluorescence Observations of Eukaryotic Translation
Nathalie Westbrook1,2
1Institut d’Optique, Palaiseau cedex, France, 2CNRS, Paris, France.
For the last two decades, structural and single-molecule investigations of the prokaryotic ribosome have provided a better understanding of the highly dynamic nature of translation. Eukaryotic ribosome has received far less attention, most of the conclusions being extrapolated from prokaryotic data. Although both ribosomes share functional and structural features, eukaryotic ribosome contains many additional domains of unknown function. Applying single-molecule techniques to eukaryote ribosomes would clarify the function of these additional domains.

In order to study structural rearrangement of the ribosome during eukaryotic elongation, we bypass canonical initiation using an initiation factor free Internal Ribosome Entry Site (IRES) enabling elongation in the desired reading frame. Ribosomal subunits, purified from Rabbit Reticulocyte Lysate (RRL), are first bound to the IRES. Ribosome-mRNA complexes are then fixed on a microscope coverslip via the 5' end of the mRNA. All factors necessary for elongation are provided by injection of RRL using single molecule Total Internal Reflection Fluorescence (TIRF) microscopy, we monitor elongation thanks to a fluorescently labelled oligonucleotide hybridized on the mRNA. The oligonucleotide is detached by the helicase activity of a translating ribosome. The evanescent wave generated by the TIRF apparatus allows solely the detection of molecules close to the surface. In this configuration, probe detachments indicate the loss of fluorescence signals which are used to measure the distribution of translation speed. A thorough care is given to photobleaching during data analysis. Controls using antibiotics further show that the loss of fluorescence is a signature of a single ribosome activity.

1327-Pos Board B219
Deciphering the Nature of the 30S:BiP A Ribosome Complex
Ala M. Shaqra
University of Connecticut, Storrs, CT, USA.
BiP A is a member of the translational family of GTPases and is required for bacterial survival under various adverse environmental conditions. It is the only protein that simultaneously binds to two ribosomal particles. Under normal growth conditions when GTP levels are high, BiP A associates with 70S ribosomes. However, during stress or other unfavorable environmental changes, BiP A binds to 30S ribosomes. This association is driven by increased levels of guanosine tetraphosphate (ppGpp), an alarmone responsible for adaptation to adverse growth conditions in bacteria. The significance of this differential association is not understood. We identified a single site substitution in BiP A that functions to suppress the cold sensitivity of Escherichia coli (cold factor 1, IF3) deletion strain suggesting a role for BiP A in translation initiation events. Biochemical and biophysical measurements of the GTPase activity of this protein reveal that it binds ppGpp with similar affinity as wild type BiP A, binds GTP weakly and only associates with the 30S ribosomal particle. Coupled with our crystallographic data, these results indicate that this protein behaves similarly to the ppGpp-bound form of wild type BiP A. These data suggest that BiP A acts as mediator between the cellular environment and the ribosome, possibly modulating the translation initiation events in response to cell stress.

1328-Pos Board B220
Free-Energy Landscape of Ribosome Translocation Analysed using MD Simulations and Cryo-EM Density Maps
Hitoshi Ishida, Atsushi Matsumoto
Japan Atomic Energy Agency, Kyoto, Japan.
Ribosome is one of the supra-biomolecules used in the process of translating genetic information for the synthesis of polypeptides. In the course of its synthesis, two tRNA molecules move with mRNA through ribosome, changing their positions at the A (aminoacyl), P (peptidyl), and E (exit) sites. This process, called translocation of tRNAs, is catalyzed by the elongation factor G (EF-G) using energy of GTP hydrolysis. Recent results from pre-steady-state kinetic analysis and cryo-electron microscopy (cryo-EM) suggest that there is a dynamic multistep process during translocation, where large-scale structural rearrangements of both ribosome and EF-G occur. However, the dynamic mechanism of translocation is unclear at the atomic level.

We used all-atom molecular dynamics (MD) simulations to direct 70S ribosome complexed with EF-G at the post-translational state (PDB code: 2WJL) towards the translational and pre-translational states (EMDB code: EMD-1365 and EMD-1363) by fitting 70S ribosome into cryo-EM density maps. Additionally, the simulations were assisted by umbrella sampling simulations, in which biased potentials were imposed on the centers of masses of the protein molecules in the 70S ribosome, to relax the transitional conformations and to construct the free-energy landscape of the translocation. Multi-step structural changes, such as a ratchet-like motion between the small and large ribosomal subunits, and a hinge-like motion of elongation factor G (EF-G) were observed during the translocation. The free-energy landscape shows that there are semi-stable states between two stable states at the pre- and post-translational states. It was shown that a loop of nucleic acids from the large ribosomal subunit, which is located near the P- and E-sites, plays an important role in the translocation of P-RNA and E-RNA.

1329-Pos Board B221
Study of Helicase Activity of the Ribosome using Single-Molecule FRET
Yi-Lan Chen, Kai-Chun Chang, Jin-Der Wen.
Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.
RNA structures may become barriers to the ribosome during translation. In the meanwhile, this feature is adopted to regulate translation in some systems such as ribosomal framingshifting. Previous studies have shown that the ribosome itself can open RNA duplex structures. Recent experiments using optical tweezers further revealed that the ribosome opens RNA hairpin structures by using two active mechanisms. However, RNA structures under the action of ribosomes are still unclear. Therefore, we aim to use single-molecule FRET (Förster Resonance Energy Transfer) to directly observe the interaction between the ribosome and RNA structures. In this study, we use an RNA which begins with six unique codons. This RNA is paired with different DNA oligomers labeled with Cy3 or Cy5 dye on either or both of the ends. These hybrids have been measured, in the absence of ribosomes, to characterize its fluorescent and FRET aspects. Next, they will be translated by our purified in vitro translation system. Therefore, we could control each translocation step of the ribosome by adding one unique aminoacyl-RNA at a time. With this design, we will be able to observe ribosomal helicase activity when the ribosome acts on the structures at various states.

1330-Pos Board B222
Single-Molecule Study on Structural Rearrangement of the 5'UTR of rpsO Messenger RNA
Yi-Ju Wu, Cheng-Han Wu, Jin-Der Wen.
Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.
Translation initiation of mRNA can be regulated through different ways in the cell. One of the common mechanisms is to modulate the structural elements of mRNA. Escherichia coli ribosomal protein S15 (ecS15, encoded by the rpsO gene) regulates its own biosynthesis by interacting with the 5' untranslated region (5'UTR) of its cognate mRNA. When ecS15 is synthesized in excess in the cell, the protein represses translation via binding to the 5'UTR of its mRNA and blocks the ribosome from accessing the initiation site. The 5'UTR region of rpsO mRNA can fold into either a pseudoknot or a double-hairpin structure, but only the former can bind the ribosome and ecS15. The pseudoknot and double-hairpin structures exist in equilibrium in solution. While the pseudoknot form has been dissected extensively in previous studies, the function of the double-hairpin is still unknown. In this study, we manage to characterize the structural dynamics of the 5'UTR of the rpsO mRNA by using optical tweezers. This technique allows us to observe conformational change of single RNA molecules in real time. Our preliminary results show that the double-hairpin structure can be rearranged to the pseudoknot conformation. We also observed some structures other than the pseudoknot and double-hairpin, and the structures have similar unfolding transition distances as the pseudoknot. According to our present data, we suggest that the double-hairpin structure may be a necessary and key intermediate in the folding pathway to the pseudoknot and some pseudoknot-like structures can form from the 5'UTR of the rpsO mRNA, though their functions remain elusive.

1331-Pos Board B223
Rearrangements of Nascent Peptide Inside the Ribosomal Exit Tunnel
Jialin Jiang, Carol Deutsch
University of Pennsylvania, Philadelphia, PA, USA.
All proteins, from bacteria to man, are made in the ribosome and are elongated, one residue at a time, at the peptidyl transferase center (PTC). This growing peptide chain wends its way through the ribosomal tunnel to the exit port, ~ 100 angstroms from the PTC. Regulation of the movement of the peptide within the tunnel and allosteric communication along the tunnel during
DNA and RNA Structure I

1334-Pos Board B226 Energy-Tunable Quantitative Hybridization Assay
Wouter H. Braunlin1, Jens Volker1, Eric Plum2, Kenneth J. Breslauer1, 
1Rutgers University, Piscataway, NJ, USA, 2IBET, Inc., Columbus, OH, USA.

We describe here a novel molecular design approach to optimizing the sensitivity and selectivity of probe-target interactions. The methodology employs a novel hybridization assay which uses a unique class of energy tunable competitor strands (C*) that hybridize to a probe strand (P). The assay is based on competitive binding equilibria for a common probe strand (P) between such competitor strands (C*) and a target strand (T). We demonstrate that families of tunable C*P complexes exhibit enhanced discrimination between targets and mismatched targets, thereby reducing false positives/negatives. The methodology also allows quantification of target strand concentrations, a determination heretofore not readily available by conventional hybridization assays. We present solution data that establish proof-of-principle for this energy-tunable quantitative hybridization assay. It is envisioned that future practical applications of this technology will be based on surface bound/spatially resolved DNA arrays.

1335-Pos Board B227 Capillary Electrophoresis as a Probe of Counterion Condensation Theory
Nancy C. Stellwagen, Earle Stellwagen. University of Iowa, Iowa City, IA, USA.

Electrophoresis is a useful probe of DNA electrostatics, because the mobility is related to the ratio of the effective charge of the DNA to its frictional coefficient. Manning has developed a theory describing the electrophoretic mobility of DNA as a function of various experimental parameters, based on counterion condensation theory. The mobility is predicted to vary linearly with cation valence, the logarithm of ionic strength, and the logarithm of the axial charge spacing along the contour length. Since the theory contains no adjustable parameters, the dependence of DNA mobility on various experimental variables can be used to probe the fundamental correctness of the underlying theory. For double-stranded DNA, the observed mobilities vary linearly with cation valence and the logarithm of ionic strength, as predicted by the Manning theory. The calculated and observed mobilities agree within ~5% if b is equal to 1.7, the axial charge spacing of dsDNA. For single-stranded DNA, the observed mobilities vary linearly with the logarithm of ionic strength and the logarithm of the fractional charge density of the backbone, as predicted by the Manning theory. However, the calculated and observed mobilities do not agree unless the value of b decreases with increasing ionic strength, raising the question of the physical meaning of this parameter.

1336-Pos Board B228 Characterization of Aggregates Formed from Oligonucleotides in the Presence of a Surfactant
Pamela M. St John, Kathleen Westervelt, Adam Rimawi. SUNY New Paltz, New Paltz, NY, USA.

Characterization of aggregates formed from oligonucleotides of 20 to 100 bases in length in the presence of the surfactant CTAB (cetyl trimethylammonium bromide) has been performed. UV spectroscopy was used to observe changes in the absorbance spectra of single and double stranded DNA in the presence of CTAB as a function of temperature and CTAB concentration. The results indicate that the spectral changes are a result of light scattering from various size aggregates formed between DNA and CTAB when the ratio of CTAB to DNA concentration is about 0.5 or larger. Electrophoresis has been used to compare the mobilities of both double and single stranded DNA in the presence of CTAB. The results show that DNA - CTAB samples give rise to broad bands in the gel images indicative of a range of aggregate sizes. Atomic force microscopy was used to obtain a topographical view of the aggregates that were dried on single crystal silicon surfaces and reconstituted in water. The aggregates in solution were apparent in optical images and both optical and atomic force microscope images showed that the aggregates were non-spherical and that they varied in size from nanometers to microns.