motion. Here we propose a mechanism whereby electric fields emanating from channels in the stator(s) create forces acting on ion binding sites in the rotor, driving it to rotate. The model predicts a scaling law relating torque to ion motive force and number of stators, consistent with experiment. The rotor of F0 drives the gamma-subunit to rotate within the ATP-producing complex (F1), working against an opposing torque that rises and falls periodically with angular position. Drawing an analogy with the washboard potential of the superconducting Josephson junction, we compute ATP production rate vs. ion motive force, finding excellent quantitative agreement with experiment and accounting for the crossover from ATP production to consumption. Plausible mechanisms for reversing the direction of bacterial flagellar rotation become self-evident in the model. (Supported by NIH R21CA133153, TcSUH, Welch (E-1221), and THECB-ARP.)

Platform D: RNA Folding & Ribosome

44-Plat
Laser Assisted Single-molecule Refolding
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In vivo, many RNA molecules can adopt multiple conformations depending on their biological context. For example, an RNA molecule initially in a stable hairpin conformation may later interact with a second RNA molecule, thus triggering a dimerization reaction. This is the case of the HIV Dimerization Initiation Sequence (DIS) and the DsrA RNA in bacteria. It is quite common that the initial interaction between the two RNAs takes place via complementary unpaired regions, forming a so-called kissing complex. However, the exact kinetic mechanism by which the two RNA molecules reach the dimerized state is still not well understood.
To investigate the refolding energy surface of RNA molecules, we have developed new technology based on the combination of single molecule spectroscopy with laser induced temperature jump kinetics, called Laser Assisted Single-molecule Refolding (LASR). LASR enables us to induce folding reactions of otherwise kinetically trapped RNAs at the single molecule level, and to characterize their folding landscape. Single molecule time trajectories show that we can drive the dimerization reaction between two trapped kissing RNA hairpins with LASR and use this data to calculate folding enthalpies and entropies. Our LASR experiments indicate that the RNA kissing complex is a stable intermediate state that facilitates the dimerization reaction.
LASR provides an exciting new approach to study molecular memory effects and kinetically trapped RNAs in general. LASR is readily applicable to study DNA and protein folding as well.

45-Plat
Single Molecule Analysis of Group I Ribozyme Folding Reveals Pronounced Ruggedness Throughout Its Folding Landscape
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It is well established that biological activity of macromolecules is intimately related to their structures. Significant efforts are directed at characterizing macromolecular structures and structure-function relationships. It is less appreciated that because of their complexity macromolecules are likely to fold not into unique structures, but into ensembles of different conformations distinct in their biological activity. Strong evidence for such complex behavior has been recently obtained in several macromolecular systems, mostly by single molecule methods. Understanding biological activity of macromolecules requires detailed characterization of the species that co-exist in the ''folded'' states, in other words - mapping of their folding energy landscape.
We used single molecule FRET to map folding energy landscape of a catalytic RNA - ribozyme derived from a group I intron. Labeling several different positions within the ribozyme allowed us to probe different parts of the folding landscape: from essentially unfolded to fully folded ribozyme. These experiments revealed broad heterogeneity of folding behaviors of individual molecules. Strikingly, even in conditions in which the ribozyme is fully folded and active, different conformations retain distinct activity. Distinct folding behaviors are ''remembered'' by individual molecules upon long incubation in conditions in which the ribozyme lacks most of its tertiary structure. This finding suggests that the origins of heterogeneity might appear at the level of secondary structure. Pinning down the structural basis for ruggedness of RNA folding landscapes and understanding its role in biology represents an exiting future challenge.

46-Plat
Identifying Energy Barriers in RNA Folding Through Kinetic Model Enumeration
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Large RNA molecules are known to fold through multiple parallel pathways to achieve their functional conformation. These pathways include stable intermediate structures that we identify using our KinFold algorithm (http://simtk.org/home/kinfold) for the analysis of time-resolved hydroxyl radical footprinting experiments. KinFold enumerates kinetic model topologies to determine the structure, lifetimes, and abundance of intermediates along the folding pathways of RNA. We are therefore able to identify the best fitting kinetic model for each folding reaction studied. We used this approach to study the folding of the L-21 T. thermophila group I intron, a catalytic ribozyme, at 6 temperatures ranging from 25° to 51° C. Our analysis of this data reveals that the rates for a majority of the transitions (between intermediate, folded and unfolded states) obey the Arrhenius equation, allowing us to estimate the relative energies of activation for inter conversion between the states of an RNA. Furthermore, we are able to estimate the lifetimes of the different intermediate structures. These results reveal a simple landscape, where folding of the peripheral elements of the RNA create significant barriers to folding. Our approach is unique in that it allows us to simultaneously estimate the activation energies for all transitions in an RNA folding reaction.

47-Plat
Uncovering The Mechanism Used By Drosophila Snf Protein To Specifically Bind Two RNAs
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The Drosophila SNF protein is a natural chimeric protein that evolved from the metazoan U1A and U2B'' proteins. In the fly, SNF is found in both the U1 and U2 snRNPs, where it is assumed to bind to the U1 snRNA Stemloop II (the site of U1A binding) and U2 snRNA Stemloop IV (the site of U2B'' binding), respectively. Like U1A and U2B'' proteins, SNF has two RNA Recognition Motifs (RRM). By analogy to U1A protein, the N-terminal RRM should bind RNA, while the C-terminal RRM does not. Our experiments show that indeed the SNF C-terminal RRM does not bind RNA; NMR data show it to be soluble and stable. The SNF N-terminal RRM alone is not stable, however, and requires the C-terminal domain to maintain a folded form. NMR experiments and ^{15}N-relaxation data show that the N-terminal RRM undergoes conformational exchange on the chemical shift timescale, while the C-terminal RRM has uniformly high order parameters characteristic of a more rigid protein.
We hypothesize that the increased flexibility of the N-terminal RRM is part of its RNA binding mechanism, since SNF does indeed bind both U1 snRNA Stemloop II and U2 snRNA Stemloop IV. The affinity of SNF for these two RNAs differs by three orders of magnitude, indicating that one is preferentially bound. By comparison, however, the affinities of the human U1A protein for these two RNAs differ by more than 10^6-fold, indicating that SNF has found a way to bind both RNAs. This hypothesis will be tested by mutation of the N-terminal domain, with subsequent determination of protein structure, dynamics, and RNA binding.

48-Plat
Parallel Pathways in 30S Ribosome Assembly
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The bacterial 30S ribosomal subunit self-assembles in vitro to form an 850kDa RNP. Assembly is generally thought to advance when the core 16S rRNA navigates through many low-energy kinetic traps, guided by the 20 small-subunit proteins that recognize and lock in native RNA tertiary structure. Kinetic analyses of protein binding reveal a highly choreographed, ordered assembly process, consistent with large-scale qualitative observations of ordered protein binding and measurements of thermodynamic cooperativity within synthetic fragments of the 30S. However, the precise order of events in this complex process are not well understood.
We have constructed a 2-photon, 3-color detection Fluorescence Correlation Spectroscopy (FCS) microscope to measure the kinetic cooperativity in the assembling 30S ribosome. Up to three ribosomal proteins of interest can be labeled with different fluorophores, and the populations of up to seven different bound species with different combinations of labels can be discriminated by

auto- and cross-correlation. We are focusing on determining the flux through parallel assembly pathways in the 3′-domain of the 30S subunit. Binding rates of proteins to the full-length 16S rRNA were obtained from FCS data, with 1-second resolution.
Assembly of the 3′-domain is initiated by binding of protein S7, followed by parallel binding of three proteins: S9, S13, and S19. These known dependencies are thermodynamic, and there is no information about the flux of the assembling ensemble through these parallel pathways. To begin to develop a kinetic map for 30S assembly, we initiated these fluorescence studies of the early 3′-domain assembly. FCS has provided measurement of the binding rate for individual proteins, and Two and Three-Color FCS spectra provides an as-yet qualitative look at the evolution of multiple intermediates, and a glimpse at how 30S assembly can proceed in parallel.

49-Plat
Visualizing tmRNA after its accommodation in the Ribosome
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In eubacteria, translation of an mRNA that lacks a stop codon produces defective polypeptide that stalls on the ribosome. Transfer-messenger RNA (tmRNA), a molecule in eubacteria that possesses functions of both mRNA and tRNA, rescues the stalled ribosome by ''trans-translation,'' a process by which the tmRNA is recruited to the ribosome with the help of EF-Tu and small protein B (smpB). Tanslation is resumed on the open reading frame of the mRNA-like domain (MLD) of the tmRNA. Several structures of tmRNA in complex with ribosome, in the accommodating and the accommodated states, have been studied by cryo-EM single-particle reconstruction (Gillet et al., 2007; Kaur et al., 2006; Valle et al., 2003). However, the structures of the complex in the subsequent stages of trans-translation remain unknown. Here, using mutagenesis, we have been able to trap the complex in different stages of trans-translation by substituting one of the sense codons of the MLD open reading frame with a stop codon. Structures of these complexes were obtained by the cryo-EM single particle reconstruction technique. To address the sample heterogeneity, we used the maximum-liklihood classification method (Scheres et al., 2007). The resulting density maps were analyzed by rigid body fitting in combination with biochemical data. We discovered that part of the tmRNA molecule maintains a relative defined structure during tran-translation. Also, we identified several possible binding sites of the tRNA like domain (TLD) of tmRNA and smpB on the ribosome.
Gillet et al. (2007) J. Biol. Chem. 282: 6356-6363.
Kaur S. et al. (2006) Proc. Natl. Acad. Sci. USA 103:16484-16489.
Scheres S. et al. (2007) Nature Methods 4: 27-29.
Valle M. et al. (2003) Science 300: 127-130.

50-Plat
Coupling Of Ribosome And tRNA Dynamics During Translation
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Comparisons of X-ray crystallographic and cryogenic electron microscopic structures of ribosomal complexes have led to the hypothesis that conformational dynamics of the ribosome, its transfer RNA (tRNA) substrates, and associated translation factors play important mechanistic and regulatory roles throughout all stages of protein synthesis. Using fluorescently-labeled components within a highly-purified in vitro translation system, we are directly characterizing structural changes of the translational machinery in real time using single-molecule Förster resonance energy transfer (smFRET) in order to elucidate the mechanisms through which these dynamics direct and regulate the individual steps of translation. Here we report new ribosome-ribosome, ribosome-tRNA, and tRNA-translation factor smFRET signals that have allowed us to fully characterize the intrinsic conformational dynamics of a ribosomal domain, the L1 stalk, as well as the coupling between L1 stalk and tRNA dynamics, throughout protein synthesis. Our data reveal that the translating ribosome can spontaneously and reversibly fluctuate between two global conformational states, and that transitions between these two states involve coupled movements of the L1 stalk and ribosome-bound tRNAs, accompanied by ratcheting of the ribosomal subunits. Furthermore, we find that elongation, release, and ribosome recycling factors uniquely recognize these global states of the ribosome and differentially affect transition rates between the two states. Thus, translation factor-mediated recognition and control over intrinsic dynamics of the ribosome plays a major mechanistic role during the elongation, termination, and recycling stages of translation. Our results support the view that specific regulation of the global state of the ribosome is a fundamental characteristic of all translation factors and a unifying theme throughout protein synthesis.

51-Plat
Regulation of the Protein-conducting Channel by a Bound Ribosome
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The evolutionarily conserved protein-conducting channel, or translocon, is a transmembrane protein which has the dual functions of allowing nascent proteins to cross the membrane or to insert into the membrane. These functions are carried out in concert with a partner which feeds the nascent protein into the channel. In many cases, this partner is the ribosome. The specific interactions between ribosome and protein-conducting channel have recently come into focus due to the availability of cryo-electron microscopy maps of the ribosome in complex with a channel monomer. We have used a method recently developed in our lab, molecular dynamics flexible fitting (MDFF), to fit atomic-scale structures into these maps. Using our fitted atomic-scale model of the ribosome-channel complex, we have carried out large (2.7 million atoms) equilibrium molecular dynamics simulations in order to investigate how the ribosome induces channel opening, as suggested by recent experiments. We find that the channel-blocking plug becomes more mobile under the ribosome's influence. By performing simulations of protein translocation through the ribosomal protein exit tunnel and into the translocon channel, we have determined what elements of the ribosome interact most strongly with the nascent chain and in what orientation the growing protein inserts into the channel.

Platform E: Excitation-Contraction Coupling

52-Plat
Impaired Sarcoplasmic Reticulum Calcium Release In Skeletal Muscle Fibers From Myotubularin-Deficient Mice
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X-linked myotubular myopathy (XLMTM) is a disease characterized by severe skeletal muscle weakness leading to death during childhood. XLMTM results from mutations in the *MTM1* gene, coding for Myotubularin, a phosphoinositide phosphatase believed to play a role in plasma membrane homeostasis. The mechanisms responsible for muscle function impairment in XLMTM are unknown. Here we studied the properties of excitation-contraction coupling in skeletal muscle fibers isolated from a mouse model of the disease. Experiments were performed under silicone-voltage-clamp conditions using indo-1 as Ca^{2+} indicator. In muscle fibers from 5-week-old *MTM1*-deficient mice, the amplitude of the voltage-activated Ca^{2+} transient was strongly reduced. For instance, in response to a 10 ms-long pulse from -80 to +10 mV, the peak $\Delta[Ca^{2+}]$ was 0.52 ± 0.1 μM (n=14) in *MTM1*-KO fibers as compared to 1.4 ± 0.14 μM in WT fibers (n=14). Conversely, the rate of $[Ca^{2+}]$ decay after the end of the pulses was similar in the two strains suggesting overall preserved myoplasmic Ca^{2+} removal capabilities. The SR calcium content was also found to be unaltered, as estimated from indo-1 signals measured in fibers equilibrated with high intracellular EGTA and in the presence of a SR Ca^{2+} pump blocker. The reduced amplitude of the Ca^{2+} transient in *MTM1*-deficient fibers was associated with a twice reduction in the peak density of the voltage-activated slow Ca^{2+} current with no apparent concurrent change in the density of intramembrane charge movement. Finally, confocal imaging with di-8-anneps revealed local disruptions in the typical fluorescence banded pattern, indicative of alteration of t-tubule membrane. Overall results unravel a critical role of MTM1 in the proper function of E-C coupling and strongly suggest that defective RyR1-mediated SR Ca^{2+} release is responsible for the failure of muscle function in myotubular myopathy.

53-Plat
Changes of EC-coupling and RyR Calcium Sensitivity in Dystrophic mdx Mouse Cardiomyocytes
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