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
Jie Fu[1], Jianlin Lei[2], Iowna Wower[3], Jacek Wower[3], Joachim Frank[4,5].
[1]Department of Biomedical Sciences, State University of New York at Albany, Albany, NY, USA, [2]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA, [3]Department of Animal Sciences, Auburn University, Auburn, AL, USA, [4]Department of Biochemistry and Molecular Biophysics, and Department of Biology, Columbia University, New York, NY, USA, [5]Howard Hughes Medical Institute, New York, NY, USA.
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
Ruben L. Gonzalez, Jr., Jingyi Fei, Samuel H. Sternberg.
Columbia University, New York, NY, USA.
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
James C. Gumbart, Leonardo G. Trabuco, Elizabeth Villa, Eduard Schreiner, Christopher B. Harrison, Klaus Schulten.
University of Illinois, Urbana-Champaign, Urbana, IL, USA.
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
Norbert Weiss[1], Lama Al-Qusairi[2], Celine Berbey[1], Bruno Allard[1], Jean Louis Mandel[2], Jocelyn Laporte[2], Anna Buj-Bello[2], **Vincent Jacquemond**[1].
[1]University Lyon 1, UMR CNRS 5123, Villeurbanne, France, [2]IGBMC, INSERM U596, CNRS UMR 7104, University Louis Pasteur, Illkirch, France.
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
Nina D. Ullrich[1], Mohammed Fanchaouy[1], Konstantin Gusev[1], Eva Polakova[2], Natalia Shirokova[2], Ernst Niggli[1].
[1]Department of Physiology, Bern, Switzerland, [2]Department of Pharmacology and Physiology, UMDNJ, Newark, NJ, USA.