requires remodeling of multiple, spatially distant structural components of the machine. In order to function efficiently, therefore, molecular machines likely must allosterically coordinate numerous, seemingly independent conformational rearrangements. Due to the significant technical challenges associated with characterizing their structural dynamics, however, the questions of whether and how large molecular machines coordinate such dynamics so as to maximize the efficiency with which they perform their biological functions remain exceptionally challenging to answer. Using a combination of structural and phylogenetic analyses, molecular genetics, single-molecule fluorescence resonance energy transfer, and in vitro biochemical assays, here we demonstrate that the ribosome uses cooperative conformational changes to maximize the efficiency with which it translocates and ejects its transfer RNA adaptors during protein synthesis. Interpretation of our data within the context provided by atomic-resolution ribosome structures and phylogenetic analyses of ribosomal RNA and ribosomal protein sequences leads us to propose a structure-based model for the observed cooperativity. Our results demonstrate that large, multi-component, molecular machines such as the ribosome can use networks of cooperative conformational changes to facilitate mechanical processes that would otherwise limit their catalytic rates.

1214-Plat
Rotational Motions of Domains in Elongation Factor G Detected by Single-Molecule Polarized Fluorescence Microscopy
Chunlai Chen[1], Xiaonan Cui[1], John F. Beausang[1], Barry S. Cooperman[2], Yale E. Goldman[1].
[1]Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA.
During the elongation cycle of protein synthesis, translocation of tRNAs and mRNA is catalyzed by the GTPase elongation factor G (EF-G) with high precision and speed. Conversion of the GTP to the GDP form of EF-G is considered essential for translocation, but the structural dynamics on the ribosome have not been reported. We used single molecule polarized total internal reflection fluorescence (polTIRF) microscopy to characterize tilting and rotational fluctuations within specific domains of EF-G. When EF-G binds to the ribosomal pre-translocation (PRE) complex, domains I and IV of EF-G undergo small rotations (10-15°) in conjunction with translocation, whereas domain III shows a much greater angular change, averaging 50°. Viomycin (Vio), which prevents translocation, reduces the rotational motions of domain III to 10-15° but has virtually no effect on the other domains. Spectinomycin also reduces domain III motions but less strongly than Vio. EF-G binding to ribosomal initiation complexes lacking A-site tRNA gives a similar pattern of domain rotations, but with shorter dwell times. In this case, the large rotation of domain III is barely inhibited by Vio. Irrespective of completion of translocation or presence of A-site tRNA, the initial 10-15° rotations of EF-G domains I, III and IV in the ribosome/EF-G complex indicate that the EF-G initially shifts the minimum of the free energy profile in the direction of translocation, suggesting that EF-G generates a force on the ribosome and/or the mRNA and tRNAs. Near the end of translocation, domain III completes its rotation either to push the mRNA and tRNAs (a working stroke) or to prevent reversal of translocation driven by thermal fluctuations (a ratchet). Supported by NIH grant GM080376 to YEG and BSC and AHA fellowship 12POST8910014 to CC.

1215-Plat
Refining Crystal Structures Against Cryo-EM Data using Molecular Dynamics Simulations to Obtain a Complete Atomistic Pathway of Transfer RNA Translocation
Andrea C. Vaiana, Carsten Kutzner, Lars V. Bock, Christian Blau, **Helmut Grubmuller**.
Theoretical and Computational Biophysics Department, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.
The movement of tRNAs during translocation is accompanied by large conformational changes of the ribosome such as intersubunit rotations. Here, we present a method to cover conformational changes of the ribosome that occur on timescales not accessible to equilibrium Molecular Dynamics (MD) simulations through the combination of X-ray crystallography, cryo-EM data and MD simulations. Cryo-electron microscopy (Cryo-EM) provides medium/low-resolution density maps for many intermediate states of large molecular complexes. In contrast, X-ray crystallography provides high-resolution structures, usually limited to few states. To obtain pathways connecting the intermediate states of tRNA translocation, we start MD simulations from crystal structures with an additional biasing potential. This biasing potential, which maximizes the correlation between atomic model and cryo-EM map (Tama 2008), allows us to drive the ribosome from one intermediate state to another, covering the full translocation pathway. This method of cryo-EM driven MD was implemented in the high-throughput and highly parallel MD simulation package GROMACS.

1216-Plat
The Effect of Codon Translation Rates on Cotranslational Protein Folding Mechanisms of Arbitrary Complexity
Edward P. O'Brien.
Cambridge University, Cambridge, United Kingdom.
Bacterial cells use the naturally occurring variability in the rate at which different codons are translated to guide the folding of nascent proteins into ordered, biologically-active structures during their synthesis by the ribosome. Predicting how codon translation rates effect cotranslational protein folding mechanisms is therefore of fundamental biological interest. Here, we demonstrate that cotranslational folding mechanisms sampling an arbitrarily large number of states can be accurately modeled by treating this problem using the Markov chain formalism. This allows a general equation to be derived that describes the probability that a nascent protein is in any one of these conformational or thermodynamic states as a function of translation rates of individual codons in an mRNA molecules' open reading frame, which we show is accurate in modeling molecular dynamics simulations of cotranslational folding. Using this framework we demonstrate that there exists scenarios in which, contrary to conventional wisdom, fast-translating codons can actually increase the amount of cotranslational folding that occurs. This approach can be applied to the cotranslational folding of cytosolic and membrane proteins, and possibly the processing of nascent chains by auxiliary factors such as chaperones and enzymes.

1217-Plat
Protein Synthesis by Ribosomes: Mapping In Vitro onto In Vivo Rates
Sophia Rudorf[1], Michael Thommen[2], Marina V. Rodnina[2], Reinhard Lipowsky[1].
[1]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, [2]Max Planck Institute of Biophysical Chemistry, Goettingen, Germany.
All living cells rely on ribosomes, powerful nanomachines that synthesize proteins by translating the information encoded in mRNA molecules. Over the past two decades, the various substeps of the translation process have been studied in much detail using in vitro systems, but it is often questioned to what extend these results can be applied to real living cells. Although in vivo translation should proceed via essentially the same steps as in vitro, the average protein synthesis rate turns out to be much faster. This difference in synthesis rate has led to a long and controversial debate because - in contrast to in vitro systems - it has not been possible to study individual conformational transitions of the translating ribosome in vivo. Here, we address this long-lasting and unresolved puzzle and introduce a general computational scheme, which enables us to map in vitro onto in vivo rates. Using new experimental data on in vitro translation at 20°C and 37°C, we first derive a complete set of in vitro rates for the individual substeps of translation. We then use our scheme, together with available in vivo data, to determine the corresponding in vivo rates. As a result, we obtain a comprehensive description of in vitro and in vivo translation for various experimental and growth conditions. This description allows us to predict codon- and mRNA-specific translation rates, which can be used, e.g., to investigate translational pauses and ribosome traffic.

Symposium: Molecular Basis of Voltage Dependence

1218-Symp
Thermodynamic Analysis of Voltage-Sensing Mechanisms
Baron Chanda.
University of Wisconsin-Madison, Madison, WI, USA.
Voltage-gated ion channels (VGIC) form a large superfamily of ion channels and the activation of these channels underlie electrical and chemical signaling in a variety of cell types. Structure-function studies are widely used to deduce the energetic effects of a mutation by measuring macroscopic currents and fitting their voltage-dependence to a Boltzmann function. However, in absence of detailed kinetic models, this approach can introduce serious errors in free-energy estimates because of the inherent assumption that the channel activation is a two-state process. We recently developed analytical tools that allows us to calculate the free energies required for activation of voltage-dependent processes without any prior knowledge of the underlying gating scheme. Our method involves measurement of conjugate displacement associated with the force that drives the activation of these channels. In the case of voltage-gated ion channels, gating charge movement is the conjugate displacement and the force is voltage across the membrane. We show that by measuring the median voltage of charge transfer, VM, and the total gating charge per channel, we can calculate the chemical free energy difference between the resting and activated state of the channels. These free-energy measurements can be extended to other members of the VGIC superfamily to obtain a measure of interaction energies