In classical folding studies folded proteins are initially denatured into an unfolded state before the (re-) folding process can be studied. It has been demonstrated that protein folding takes place already during the elongation of the nascent chain (co-translational folding).
Here we present an approach employing a two color single molecule sensitive fluorescence wide-field microscope in order to visualize surface tethered fluorescently labeled ribosomes and *de novo* synthesized GFP molecules in real time [1]. Suppression of protein release after synthesis keeps the synthesized GFP bound to the ribosome and allows to image GFP fluorescence for extended observation times.
We demonstrate that the characteristic time for the production of the mature GFP mutant Emerald (GFPem) is five minutes, which is one of the fastest maturation times for a GFP mutant observed so far. Early GFPem molecules appear even faster, within one minute. Processes precedent to chromophore formation, such as polypeptide synthesis and protein folding, are fast and last less than one minute. Thus cellular processes within a time range of a few minutes can be followed by GFPem.
[1] A. Katranidis et al. (2009)Angewandte Chemie Int. Edit., **48**, 1758-1761

1355-Pos
Fluctuating tRNA Guided by Induced Fit as a Basis of High Fidelity Translation by the Ribosome
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Positional fluctuations and structural flexibility of molecules play important roles in various cellular processes. We implemented a single molecule method that can simultaneously measure fluorescence resonance energy transfer (FRET) and the anisotropy of FRET acceptor emission. Based on the method, we monitored the process of aa-tRNA selection by the ribosome and evaluated the positional fluctuations of a cognate and a near-cognate tRNA at the GTPase activated state. The FRET measurements revealed that a cognate tRNA at the GTPase activated state samples the ''A'' site of the ribosome more frequently than a near-cognate. A cognate tRNA is found fluctuating more slowly within a more restricted space as compared to a near-cognate. These results suggest that induced fit steers a fluctuating cognate tRNA more accurately toward the ''A'' site of the ribosome. Based on our findings, we propose that fluctuating tRNA guided by induced fit may be the basis of high efficiency tRNA selection by the ribosome.

1356-Pos
Simulations of the Bacterial Ribosomal Decoding Switch
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Gentamicin is a potent antibiotic often used in therapy for methicillin-resistant Staphylococcusaureus. Gentamicin works by flipping a conformational switch on the ribosome, disrupting the reading head (i.e., 16S ribosomal decoding bases A1492-A1493 used for decoding messenger RNA. We use explicit solvent all-atom molecular simulation to study the thermodynamics of the ribosomal decoding site and its interaction with gentamicin. The replica exchange molecular dynamics simulations allow enhanced sampling of the unbinding free-energy landscape, including a rigorous treatment of enthalpic and entropic effects. The decoding bases flip on a timescale faster than that of gentamicin binding, supporting a stochastic gating mechanism for antibiotic binding, rather than an induced-fit model where the bases only flip in the presence of a ligand. The study also allows us to explore the nonspecific binding landscape near the binding site and reveals that, rather than a two-state bound/unbound scenario, drug dissociation entails shuttling between many metastable local minima in the free-energy landscape. Additional simulations address the effect of mutation/modification of the A-site on the free energy landscapes. In particular, mutations of base A1408 known to confer high-level resistance in ''superbug'' bacteria to common antibiotic therapies are investigated. Special care is dedicated to validation of the obtained results, both by direct comparison to experiment and by estimation of simulation convergence.

1357-Pos
Single-Molecule Study of Programmed Ribosomal Frameshifting
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Programmed ribosomal frameshifting is involved in regulation of gene expression at the translation level in bacteria, and the frameshifting efficiency has to be well controlled. For example, the dnaX gene of *E. coli* encodes two subunits (gamma and tau) of the DNA polymerase III, and the ratio of these two subunits is determined by the frameshifting efficiency. Factors that affect frameshifting have been extensively studied in vitro and in vivo, but the dynamic features of this process are still not well understood. Here we use optical tweezers to follow stepwise translation in real time at the single ribosome level to understand how frameshifting is controlled. Our preliminary data show that the ribosome pauses for various times at, or even one to two codons prior to, the slippery sequence where frameshifting occurs. The pause may be caused, at least in part, by an internal Shine-Dalgarno sequence, which is located upstream to the slippery sequence and is known to induce frameshifting. Correlation between pause duration and occurrence of frameshifting is under investigation.

1358-Pos
Single-Molecule Optical-Tweezers Studies of Ribosome Translation and Unwinding of Messenger RNA
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Ribosomes translate the genetic code in an mRNA into a protein; three nucleotides-one codon-code for one amino acid. Because natural mRNAs contain base-paired regions, the ribosome needs to unwind these structures into single-strands before the structured regions can be translated. Furthermore, mRNA secondary and tertiary structures are involved in translation regulation mechanisms, such as frame-shifting. Bulk studies have shown that mRNA structures slow down the translation rate and that the unwinding is inherent to the ribosome. But the unwinding mechanism is not well understood. We used optical tweezers to apply force to the ends of an mRNA hairpin being translated by a single ribosome. At constant force, the mRNA end-to-end distance increases as the ribosome translates the message and converts double-stranded RNA into single strand. This technique allows observation of codon-by-codon translation. The translation rate dependence on force provides information on the ribosome unwinding mechanism. We found that the translation rate increases quickly as force is increased within a narrow force range, but the rate plateaus below and above this force range. We also found that the translation rate on duplex mRNA (low force plateau) is ~50% of the rate on single-stranded mRNA (high force plateau). The observed force dependence is inconsistent with a passive unwinding model, or the active unwinding models that have been applied to T7 and NS3 helicases. Instead, the data call for a different type of active unwinding mechanism. This result suggests that there might be fundamental differences in the unwinding mechanism of ribosomes and of other nucleic acid helicases. Our results show a tight coupling of the ribosome translation and unwinding activities, and should facilitate understanding of translation regulation mechanisms, such as frame-shifting.

1359-Pos
Insights into Translational Termination from Crystal Structures of the 70S Ribosome Bound to Release Factor
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Here we report high-resolution crystal structures of release factor 2 (RF2) bound to 70S ribosome. These structures emulate the translational states directly before and after hydrolysis of the ester bond in peptidyl-tRNA on the ribosome during translational termination. Our structures show detailed molecular interactions between the ribosome decoding center and RF2 upon stop-codon recognition. After a stop-codon is recognized, the universally conserved GGQ motif extends directly into the peptidyl transferase center forming a tightly packed catalytic core. Nucleotide A2602 in the 23S rRNA forms a favourable stack with the GGQ motif in RF2. The ribose of the A76 in the peptidyl-tRNA adopts C2'-endo conformation. Our structures help to rationalize a decade of biochemical and computational data on translational termination. Based on the structures, a mechanistic model on how the ester bond in the peptidyl tRNA is hydrolyzed is proposed.

1360-Pos
Realtime Observation of tRNA Dynamics at High Concentrations in Single Molecule Translation
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Conventional TIRF methods can probe the dynamics of complex biological systems, but only at concentrations of fluorescent components that are less