Investigating Ribosome Conformations with Multi-Resolution Modeling
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In the past years, crystallography and cryo-electron microscopy have brought us many structures of the ribosome in various conformations. These have led to new insights on translation. However the dynamics connecting states are not well understood, due to the difficulty of observing transient events in atomic detail. This motivates molecular modeling as a tool to obtain such information. In our work, we perform internal coordinates simulations with our program MMB (Macro Molecule Builder) to generate highly detailed structures and trajectories still not observed experimentally.

This type of simulation permits us to selectively rigidify parts of the molecules, allowing large conformational movements while maintaining physical atomic interactions. It is possible to define limited physics zones, where we apply a standard Molecular Dynamics force field at low cost.

A novel interactive 3D Graphical User Interface makes once-formidable modeling tasks accessible to any scientist. The package facilitates threading, morphing, flexible fitting, design of mutations, and many other tasks. We have applied this to resolve all-atoms trajectories of ribosomal translocation and in collaboration with crystallographers, we generated conformations for peculiar structures of tRNA anticodon loop to support experimental observations in the context of –1 frameshift.

Influence of Downstream DNA/RNA Structure on Intersubunit Rotation of the Ribosome
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The ribosome, composed of two subunits (30S and 50S), is a molecular machine that translates the genetic code inscribed in the mRNA into a protein sequence through repetitive cycles of aminonucleoside-RNA selection, peptide bond formation and translocation. However, the presence of mRNA secondary or tertiary structures change the rhythm of translation and in many instances may influence reading frame maintenance. We have shown with smFRET and SAXS that mRNA structures when present outside the entry tunnel of the ribosome induce a hyper-rotated state of the 30S subunit and render the L1 stalk primarily in an open configuration. In this study, we seek to investigate the distance dependence between downstream DNA oligos and the mRNA entry tunnel of the ribosome to determine how close structures need to be to induce the rotation of the ribosome. Activities of DNA-RNA hybrids were created to assemble with fluorescently labeled ribosomes and intersubunit rotation was followed by single molecule FRET.

Exploring the Ribosome Helicase Activity in Context of Frameshifting using Single-Molecule Techniques
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Although the structure of the bacterial ribosome, with varying resolutions has been uncovered, the subtle dynamics and interactions are to be pursued further. One vital aspect is the intrinsic helicase activity of the ribosome and how it unfolds structured mRNA during translation. The relevancy of the helicase function extends beyond general translation processes into recoding mechanisms such as frameshifting. Such process is found in viruses, bacteria and eukaryotes at varying efficiencies related to both physiological and pathological functions posing an interest to explore it further. Thus, we aim to study this process using single-molecule approaches along with bulk methods. First, we are developing bifluorescent constructs to quantify frameshifting both in vitro and in vivo for bacteria. Also, we are developing an ultrahigh resolution optical tweezers with fluorescence detection capabilities. The tweezers - FRET setup along with different labeling schemes could be used to monitor the ribosome during translation and its interactions with mRNA. This could elucidate further the dynamics and conformational changes intrinsically within the ribosome during the process of unfolding. In addition, it could provide fundamental insight into the variable efficiency of different frameshifting signals whether through the thermodynamic stability or conformational changes of structured elements within the signal.

The mRNA Secondary Structure Impact on Translation of the KCNH2 Channel Protein
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The gene KCNH2 encodes the K+ channel responsible for the repolarizing IKr current essential for normal cardiac physiology. Genetic, translational and post-translational perturbation of KCNH2 causes hereditary (Locus LQT2) and acquired Long QT Syndrome. Over 600 deleterious mutations have been identified in KCNH2, most leading to protein trafficking or assembly malfunction. Investigation has focused on the impact the changed amino acids have on the protein product, but little is known about mutational effects on mRNA function. KCNH2 is unusual in that its GC content is ~66% and there is increased use of rare codons when compared to other human mRNAs. Previous studies from our lab have shown that KCNH2 channel translation and trafficking efficiencies are dependent on “extra-coding” elements in the mRNA sequence. CM-KCNH2, a codon modified version of the KCNH2 mRNA, was made with reduced GC content (51%) and decreased use of rare codons, while maintaining the exact amino acid sequence. CM-KCNH2 protein product was a more efficiently trafficked protein, but was translated less efficiently when compared to native KCNH2 (NT-KCNH2). Whether codon-usage, GC content or mRNA 2’ structure controls these processes is unknown. To address this, we will employ a combined approach of ribosomal profiling to analyze precisely where translational slowing/pausing occurs, and RNA SHAPE analysis to investigate the secondary structure of the KCNH2 mRNA. We hypothesize that the indicated sites of ribosomal pausing, caused by secondary mRNA structure and rare codon placement, allow for correct secondary and tertiary structure formation of the KCNH2 protein, indicating that mRNA impacts not only the primary structure of a protein, but through “extra-coding” features, impacts the folding efficiency and function of the protein product. Ongoing studies will have implications for evaluation and treatment of hereditary and acquired arrhythmias.

Translocation of tRNAs through the ribosome is accompanied by large-scale movements of the small ribosomal subunit relative to the ribosome. Changes in the relative interactions between the subunits have to be finely balanced to allow this high conformational flexibility while at the same time maintaining integrity of the ribosome complex. Here, we address the question of how the intersubunit interactions are fine-tuned by extensive all-atom, explicit solvent molecular dynamics simulations of the 70S ribosome starting from 13 distinct translocation intermediate models. These models were obtained by refining crystal structures against cryo-EM reconstructions of the spontaneously translocating ribosome. Analysis of the trajectories at the residue level revealed two classes of intersubunit contact interactions: i) persistent residue contacts that are independent of intersubunit rotation and primarily located close to the axis of rotation. ii) contacts that are formed and ruptured depending on the rotation angle, seen mostly on the periphery. Strikingly, also these rotation specific contacts on the periphery substantially contribute to the overall stability of the ribosomal assembly and may serve to maintain a constant interaction energy with low barriers for rotation. Our simulations revealed that upon removal of tRNAs peripheral contacts are weakened and, in turn, intersubunit rotation angles decrease. This result is supported by recent cryo-EM data of tRNA depleted ribosomes and underlines the importance of these peripheral contacts.

Simulating the Pulling of Stalled Elongated Peptide from the Ribosome by the Translocon
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The nature of the coupling between the stalling of the elongated nascent peptide chain in the ribosome and its insertion through the translocon is analyzed, focusing on the recently discovered biphasic force that overcomes the stalling barrier. The origin of this long-range coupling is explored by coarse-grained simulations that combine the translocon (TR) insertion profile and the effective chemical barrier for the extension of the nascent chain in the ribosome. Our
The Cryo-EM Structure of the Ribosome Bound to BipA of NVL2, which are dissociated from the MTR4/DOB1-containing complexes. First, HEK293 cells that can inducibly overexpress wild-type or mutated NVL2 are infected with S. enterica typhimurium 70S ribosome bound to BipA-GMPNP. Our current 9A reconstruction, while not high enough in resolution to resolve residue-nucleotide interactions, confirms that BipA’s binding site overlaps with that of EF-G. This is in accordance with biochemical assays that show competition between EF-G and BipA for binding. Additionally, the structural features of BipA in the reconstruction suggest that it has a flexible CTD and its bound form is different from the free-floating state, resembling properties of EF-G. Moreover, our structure suggests that the stabilizing role of the BipA binding to the ribosome is reinforced by the presence of an A-site tRNA. The flexibility of the CTD may aid in the formation of additional ribosomal contacts that enhance BipA’s GTPase activity. Indeed, new biochemical data reinforce these observations and may suggest the existence of a novel mechanism of translational regulation.

Quantifying the Spatial Organization of Bacterial Ribosomes using Three-Dimensional Super-Resolution Microscopy

The Cryo-EM Structure of the Ribosome Bound to BipA

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