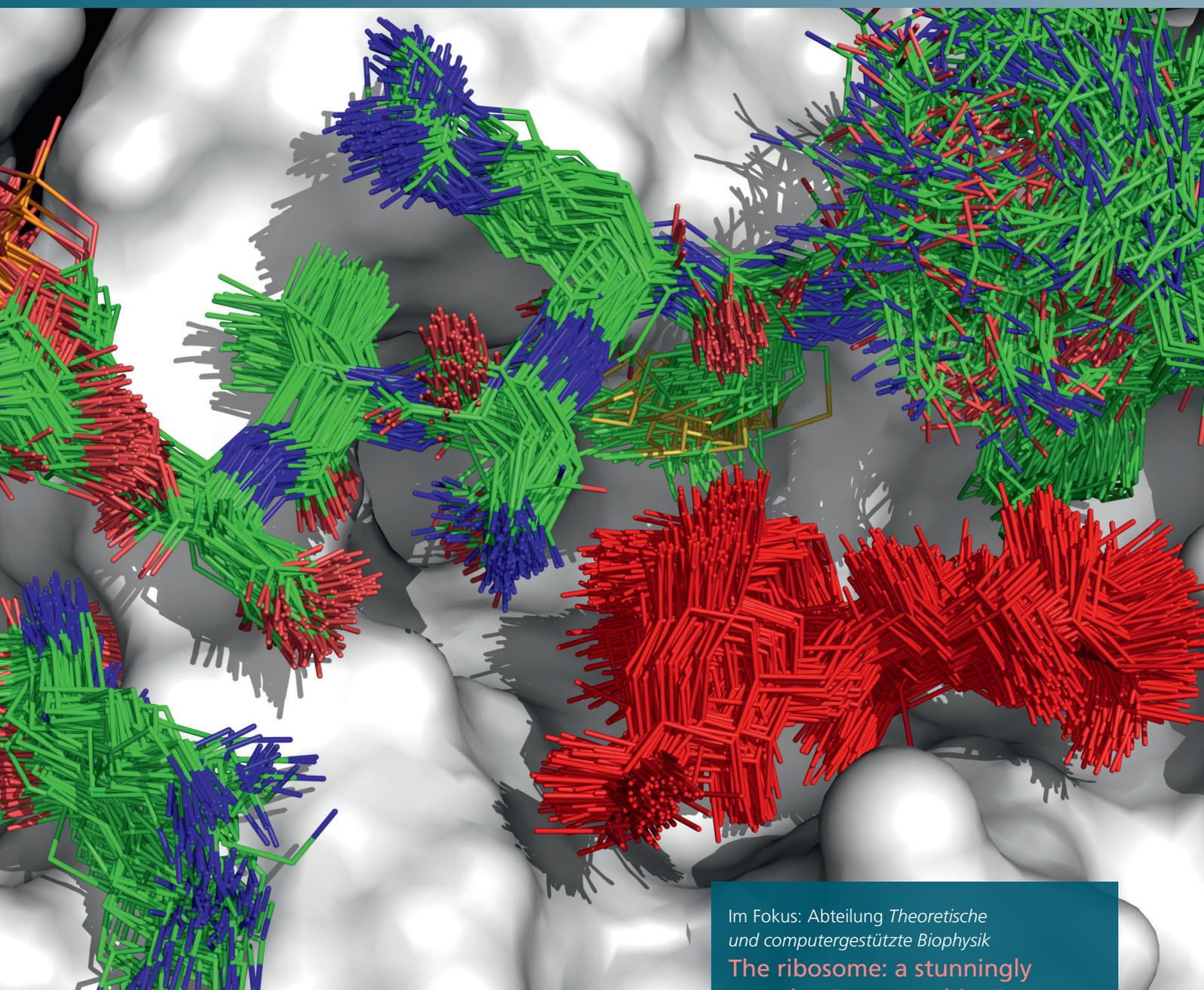




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The ribosome: a stunningly complex "nanomachine"

Nachrichten

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The ribosome: a stunningly complex “nanomachine”

Lars V. Bock, Michal H. Kolář, Andrea C. Vaiana, Helmut Grubmüller

The ribosome is a macromolecular complex that produces all proteins within the cell in a process called translation. It is thus arguably one of the most important “nanomachines” in all forms of life, ranging from bacteria, and plants to animals and humans. Bacterial ribosomes are also the target of many antibiotics. In the Department of *Theoretical and Computational Biophysics*, and in close collaboration with the *Structural Dynamics* Department headed by Holger Stark and Marina Rodnina’s *Physical Biochemistry* Department at the institute, as well as with external groups, we seek to find out how this complex machinery performs its function at the atomic level. Specifically, we would like to know which molecular forces and conformational motions cause which action, and how these are orchestrated? Or, more specifically, how precisely is the genetic code decoded and translated, what can nevertheless go wrong, and why? And how do antibiotic molecules actually block or reduce the ribosome’s efficiency?

We address these questions using atomistically resolved computer simulations as our main tool, in which the motion of the millions of interacting atoms of which the ribosome is

composed are calculated from fundamental laws of physics. This enables us both to analyze and explain functional motions of the ribosome as well as to validate our results against experiments. Vice versa, our simulations rest on accurate structural information of the ribosome provided by X-ray crystallography or cryo-electron microscopy (cryo-EM).

The ribosome (Fig. 1) is composed of ribonucleic acid (RNA, red and blue) strands and a few dozen proteins. Ribosomes read the genetic blueprint for a protein encoded in the messenger RNA (mRNA) and translate it into a chain of protein building blocks (amino acids), one per work cycle. During each cycle, one amino acid is carried into the ribosome by a transfer RNA (tRNA, yellow and green), which at its opposite side – at the decoding center (near bottom) – presents its respective genetic code to the mRNA. Only the tRNA carrying the correct code is accepted, processed further, and its amino acid is appended at the peptidyl transferase center (PTC) (further up in Fig. 1) to the growing nascent peptide chain, which leaves the ribosome through a long, winded, and narrow exit tunnel (upper right). The process of translation is regulated by several factors (for example EF-Tu, red)

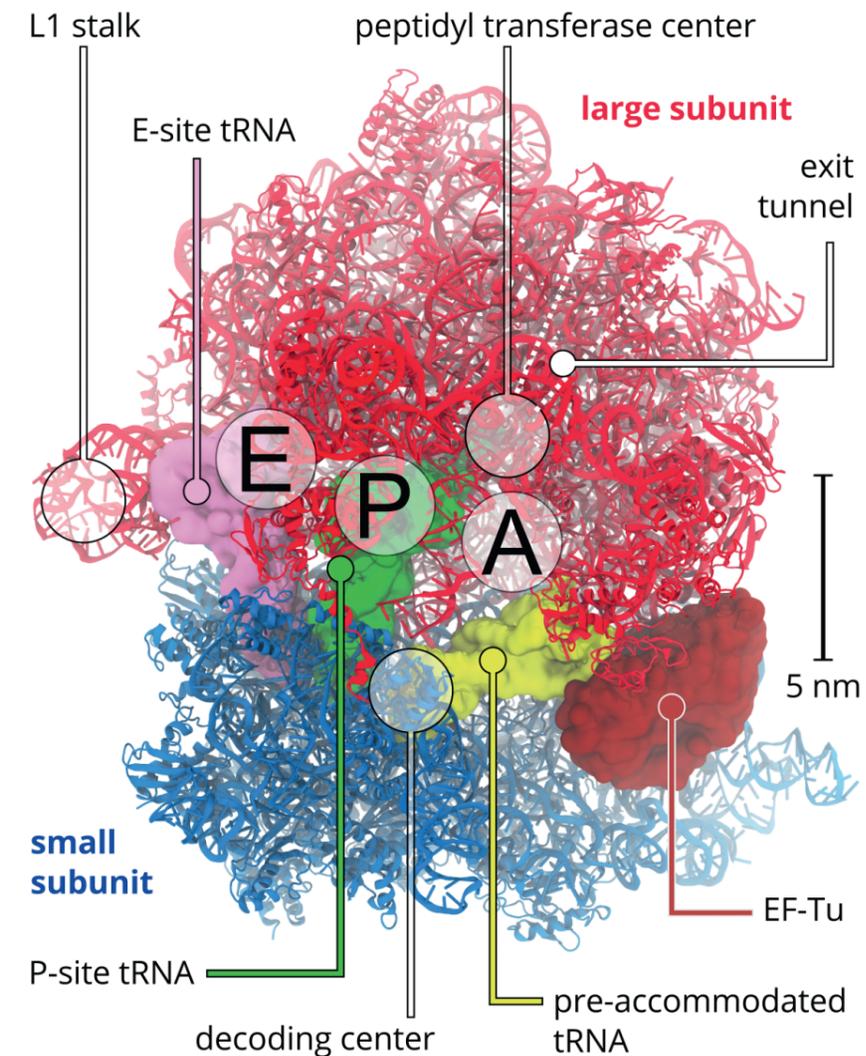


Fig. 1: Anatomy of the bacterial ribosome (adapted from [1]).

and involves cyclic binding of tRNAs into three specific sites on the ribosome (Fig. 1, labeled A, P, and E). The empty tRNA exits the ribosome through the exit site (Fig. 1, E-site). The ribosome is reset for the next amino acid by shifting the A-site tRNA with the nascent peptide to the P-site.

Our molecular dynamics (MD) simulations of the ribosome [1-3] pose significant numerical challenges, which can be tackled only by high-performance supercomputer facilities. They need to cover, for example, a wide range of length and time scales – from the size of the ribosome, which is tens of nanometers (one nanometer is the millionth part of a millimeter), down to motions as small as atomic bond vibrations (~0.01 nanometers). To resolve these motions, the simulations need to proceed in tiny time steps (each about a femtosecond, which is the 1,000,000,000,000,000th part of a second, pretty short!). However, the simulations need to cover at least part of the time span at which the much slower motions of the ribosome occur (between microseconds and milliseconds). This is the second – and most limiting – challenge in our computational approach. Despite the large computational resources provided by the Max Planck

Society, the GWDG, and the Leibniz Computer Center (Munich) for this purpose, our simulations of the ribosome take several weeks to months. Below, we will sketch some of the results we have obtained for the above questions.

(a) Translational stalling mechanism in the presence of the antibiotic erythromycin

Erythromycin is an antibiotic which binds inside the exit tunnel of bacterial ribosomes and thereby can reduce their efficiency or even block protein synthesis (stalling). One might think that the mechanism is simple: Like a cork in a bottle, the erythromycin molecule blocks the exit for the nascent peptide. However, it is not as simple: Stalling is highly sequence specific, and many sequences can pass the blockage and exit the tunnel, some without loss in efficiency. In contrast, several amino acids have been shown to be particularly vulnerable. For example, a lysine at position 11 (K11) of the ErmBL peptide causes severe stalling, for unknown reasons.

In collaboration with the *Protein Synthesis and Ribosome Structure* group of Daniel Wilson at the University of

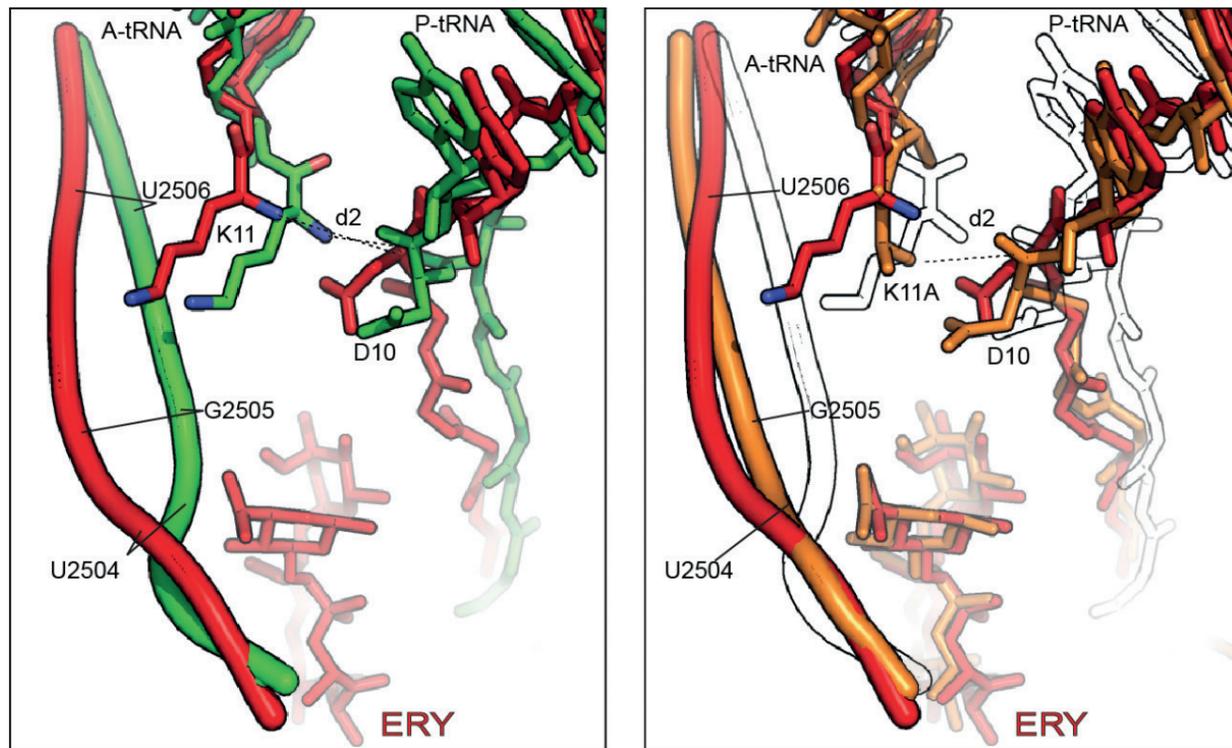


Fig. 2: Our simulations show that the antibiotic erythromycin (ERY) causes deformations of critical parts of the PTC that attaches the peptide to the growing nascent chain. Comparison of conformations obtained from MD simulations, A- and P-site tRNAs, as well as ERY are drawn as sticks and the backbone of ribosomal nucleotides (U2504-U2506) is shown in ribbon representation. The left panel compares conformations with (red) and without ERY (green). In the presence of ERY, the amino acid K11, which is attached to the A-site tRNA, moves away from amino acid D10 attached to the P-site. The right panel compares conformation with K11 mutated to alanine (orange), showing that the alanine approaches D10, thereby recovering the ability of peptide bond formation (Fig. adapted from [4]).

Hamburg, which resolved cryo-EM structures of the stalled complex, we studied the mechanism of the antibiotic-induced stalling [4] in more detail. To this aim, we simulated the complex in the presence of and after the computational removal of erythromycin, thereby complementing the cryo-EM experiments which cannot resolve the unstalled complex. In the simulations, erythromycin induces a conformational change in ribosomal nucleotides of the PTC. These negatively charged nucleotides are attracted to the positively charged amino acid K11 attached to the A-site tRNA. This attraction results in a shift of K11 away from the adjacent amino acid (D10) attached to the P-site tRNA, thus preventing peptide bond formation between the two (Fig. 2, left panel). Previously, it was also shown that a mutation of K11 to alanine prevented stalling. Indeed, when we induced this mutation also in our simulations, the uncharged alanine did not interact with the ribosomal nucleotides and, therefore, was not shifted away from the P-site tRNA (Fig. 2, right panel), thus explaining this so far puzzling experimental result. Further, the simulations predicted that a mutation of K11 to the so far untested amino acid arginine should enhance the stalling ability of ErmBL, which was subsequently confirmed by biochemical experiments [4].

(b) Rescue of stalled peptides by elongation factor EF-P

Also in the absence of antibiotics, the ribosome may run into problems. For example, if the ribosome synthesizes proteins

containing several specific amino acids – the prolines – in a row, the nanomachine is likely to stall due to the prolines' peculiar chemical properties. In bacteria, stalling is overcome by a specialized protein, the elongation factor EF-P, which contains a modified amino acid, K34. To decipher the mechanism of how EF-P alleviates stalling, we collaborated with the Wilson and Rodnina groups.

Using cryo-EM, Daniel Wilson's team managed to resolve structures of a ribosome stalled by a proline stretch both in the presence and absence of EF-P [5]. The structures with EF-P (black lines in Fig. 3) showed that the modified amino acid, with its finger-like modification (β -lys.Lys in Fig. 3), reaches into the CCA end of the P-site tRNA to which the nascent peptide is attached before peptide bond formation. The structures also show that the conformation adopted by the tRNA in presence of EF-P closely resembles the known conformation of a ribosome which is primed for peptide bond formation (grey lines in Fig. 3 – pre-attack conformation).

Our MD simulations revealed the effect of EF-P on the region around the PTC: In the presence of EF-P (green lines in Fig. 3), the P-site tRNA is stabilized in an "intact" conformation that allows peptide bond formation. Moreover, with unmodified EF-P (blue lines) or without EF-P (red lines), the P-site tRNA moves away from the amino acid attached to the A-site tRNA, thereby perturbing the conformation of the PTC machinery, which leads to stalling (Fig. 3) [5].

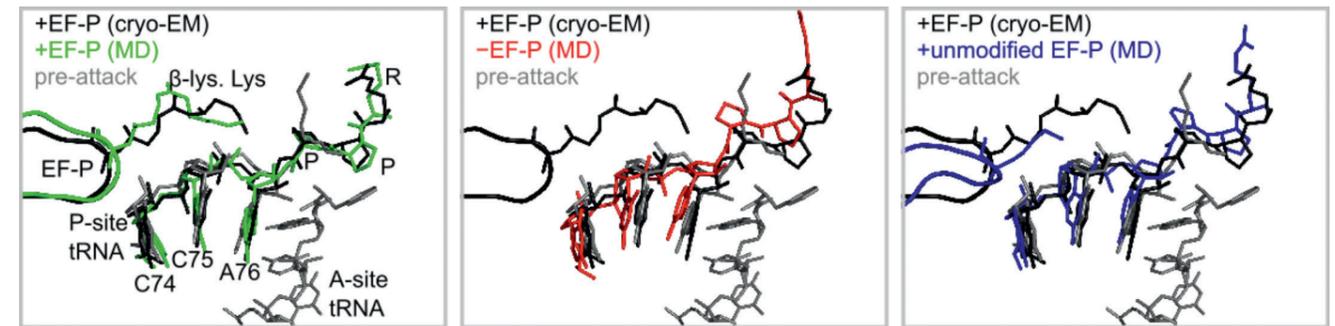


Fig. 3: Comparison of conformations obtained from MD simulations (colored) and cryo-EM (black). The modified lysine K34, CCA-tails of P- and A-site tRNAs, and the nascent peptide (PPR) are shown as sticks. In the MD simulation with EF-P (left panel, green), the conformation remains close to the cryo-EM structure. In the simulations without EF-P (center, red) and with unmodified EF-P (right, blue), the P-site tRNA moves away from the A-site tRNA, hindering the formation of a peptide bond between the A-site amino acid and P-site peptide (Fig. adapted from [5]).

(c) Peptide elongation through the exit tunnel

In an ongoing project, we are studying the structure and dynamics of growing nascent peptides in the ribosome exit tunnel. Since a peptide is quite flexible and is "pushed" at the PTC right within the ribosome's center all the way towards the outside, this task is similar to an attempt to push a cooked spaghetti through a narrow and winded channel – it is completely unclear how the ribosome accomplishes this feat.

To resolve this puzzle, we simulated this process and inserted one amino acid at a time to the P-site tRNA, thereby pushing the peptide through the tunnel. We studied three model peptide sequences, poly-phenylalanine (poly-Phe), poly-alanine (poly-Ala), and poly-glycine (poly-Gly). These sequences were also used in spectroscopy experiments by the Rodnina group, in which the peptides were labeled by a fluorescent dye (BOF), such that the precise sequence and timing of the extensions could be observed.

Our initial, only several microseconds long simulations underscored the "spaghetti problem" (Fig. 4, protein properly colored in yellow). We observed very slow relaxation of the poly-Phe peptide, such that the initial part of the tunnel was only able to accommodate about six amino acids. Interestingly, this obstacle to further translocation was sequence-specific: poly-Gly and poly-Ala peptides relaxed faster than poly-Phe, possibly due to their smaller sizes. In subsequent simulations, we were able to build a much larger peptide containing up to 16 amino acids from scratch. The pushing force generated during the first steps of amino acid addition in our simulations dissipated quickly and did not propagate to distances beyond a few amino acids. As a consequence, the peptide formed a compact fold already before entering a challenging part of the tunnel that is particularly narrow (Fig. 4, at the right side).

Already at this early stage of the project, it has become clear from our simulations that the initial pushing force exerted at the PTC does not suffice to explain the movement of the peptide further down the tunnel. Other effects, such as specific interactions between the growing peptide and the tunnel walls or entropic forces arising from the restricted thermal motion of the peptide in the tunnel, may mark the difference between nascent peptides and our cooked spaghetti.

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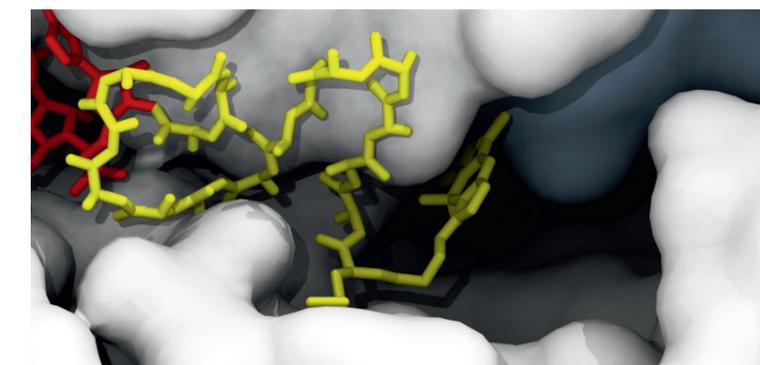


Fig. 4: The nascent poly-alanine peptide (yellow) with the fluorescent BOF dye attached at the right side initially forms a quite compact structure within the ribosome tunnel (grey surface). The P-site tRNA (red) "pushes" the nascent chain towards the right and the exit of the tunnel.