Supplementary Information

Statics of the ribosomal exit tunnel:
Implications for co-translational peptide folding,
elongation regulation, and antibiotics binding

Simone Fulle¹, Holger Gohlke¹,²*

¹Department of Biological Sciences, Molecular Bioinformatics Group, J.W. Goethe-University, Frankfurt, Germany
²Department of Mathematics and Natural Sciences, Pharmaceutical Institut, Christian-Albrechts-University, Kiel, Germany
**Figure S1:** Color-coded representation of the flexibility characteristics obtained by a flexibility analysis of the large ribosomal subunit. The coloring of the backbone atoms of the RNA part is according to the flexibility index of the glycosidic bonds and according to the Cα-Cβ bonds in the protein part. Blue color indicates overconstrained regions, red color flexible regions; isostatically rigid regions are colored in green; glycines and prolines are shown in gray.
Figure S2: Representation of the flexibility characteristics of the tunnel vicinity in terms of spatial segments obtained by diluting the H-bond network (i.e., setting $E_{HB} = -2.4$ kcal/mol for protein and RNA) in the underlying network representation. The tunnel region was split into cylindrical shells of 10 Å length parallel to the tunnel axis (abzissa) and 2 Å width perpendicular to the tunnel axis (ordinate). The color-coded representation of the shells is determined by averaging flexibility indices (Eq. 1) of P and Cα-atoms located within a shell. Stable regions are indicated by a color gradient ranging from blue ($f_i = -0.5$) to light-blue ($f_i = 0.0$); for flexible regions the gradient ranges from orange ($f_i > 0.0$) to red ($f_i = 0.5$).
Figure S3: Representation of the flexibility characteristics of the tunnel vicinity from (a) *E. coli* (PDB code 2AW4), (b) *T. thermophilus* (PDB code 2J01), and (c) *D. radiodurans* (PDB code 2ZJR) structure in terms of spatial segments. The tunnel regions were split into cylindrical shells of 10 Å length parallel to the tunnel axis (abzissa) and 2 Å width perpendicular to the tunnel axis (ordinate). The color-coded representation of the shells is determined by averaging flexibility indices (Eq. 1) of P and C\textsubscript{α}-atoms located within a shell. Stable regions are indicated by a color gradient ranging from blue ($f_i = -0.5$) to light-blue ($f_i = 0.0$); for flexible regions the gradient ranges from orange ($f_i > 0.0$) to red ($f_i = 0.5$).
Figure S4: Flexibility characteristics of nucleotides adjacent to L4 of an *E. coli* (PDB code 2AW4) structure. Overconstrained regions are indicated by blue color and flexible regions are shown in red color; isostatically rigid regions are depicted in green color. Here, opening of a side pocket was observed based by cryo-EM. Notably, nucleotides A789-A794EC adjacent to L4 are identified by constraint counting to be flexible, allowing for movements of the enclosed loop.
Figure S5: Alignment of the protein L22. The native conformation of L22 (PDB code 1S72) is shown in orange, the isolated conformation (PDB code 1BXE) in blue, and the swung conformation triggered by the binding of TAO (PDB code 1OND) in silver. The hinges Arg125 and Ala133 (*H. marismortui* numbering) are highlighted in red.
Figure S6: Flexibility characteristics of the tunnel constriction-forming proteins L4 and L22 of a *T. thermophilus* structure (PDB code 2J01). The coloring of the backbone of the tips of L4 and L22 is shown according to the values of the Cα-atoms. Overconstrained regions are indicated by blue color and flexible regions are shown in red color; isostatically rigid regions are depicted in green color. In addition, the swung conformation of L22 (extracted from PDB code 1OND after superimposing the L22 structures) is shown in silver. Numbering within the figures refers to *E. coli*. Constraint counting on the T50S structure reveals flexibility in residues Val85TT (ILE85EC) and Ala93TT (Ala93EC) of the β-hairpin tip of L22. The same hinge regions have been found by constraint counting on the H50S structure and based on an observed conformational change of L22 in a *D. radiodurans* structure (PDB code 1OND).
Figure S7: Flexibility characteristics of the tunnel penetrating protein loops (a and b) of L39e of the *H. marismortui* structure (PDB code 1S72), (c) L23 of the *T. thermophilus* structure (PDB code 2J01), (d) L23 of the *E. coli* (PDB code 2AW4) structure, and (e) L23 of the *D. radiodurans* (PDB code 2ZJR) structure. (b) is rotated by 90° with respect to (a). The coloring of the backbone of the L39e and L23 proteins is shown according to the values of the Cα-atoms. Overconstrained regions are indicated by blue color and flexible regions are shown in red color; isostatically rigid regions are depicted in green color.