

Supplementary Table and Figures for

Kinetic Mechanism and Determinants of EF-P Recruitment to Translating Ribosomes

Vitalii Mudryi, Jan Ole Frister, Bee-Zen Peng, Ingo Wohlgemuth, Frank Peske and Marina V. Rodnina

Department of Physical Biochemistry, Max Planck Institute for Multidisciplinary Sciences, 37077 Goettingen, Germany

Corresponding author: Marina V. Rodnina, e-mail: rodnina@mpinat.mpg.de; Tel: +49 5512012901.

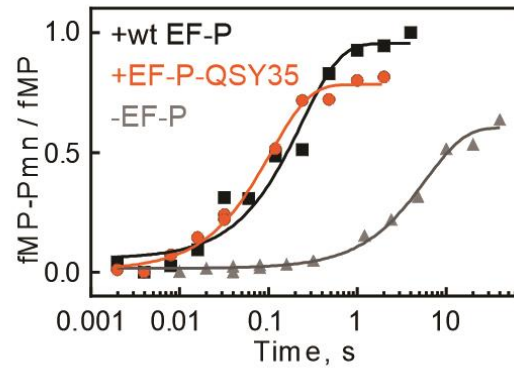
Supplementary Table 1. Kinetic parameters of EF-P binding to different ribosome complexes calculated from the concentration dependence of k_{app1} and k_{app2} .

P-site tRNA	E-site codon	β -Lys	$k_1, \mu\text{M}^{-1}\text{s}^{-1}$	k_{-1}, s^{-1}	k_2, s^{-1}	k_{-2}, s^{-1}
fMP	AUG	+	45 ± 2	8 ± 5	5 ± 6	3 ± 6
fM	AUA	+	41 ± 1	18 ± 2	10 ± 4	6 ± 3
fM (PRE)	AUA	+	34 ± 1	11 ± 3	4 ± 2	1 ± 1
fMF	AUG	+	42 ± 5	69 ± 13	8 ± 10	11 ± 5
fMC ^b	AUG	+	40 ± 8	44 ± 20	16 ± 22	13 ± 17
fMK ^b	AUG	+	40 ± 5	127 ± 35	19 ± 9	14 ± 6
fMPP	CCG	+	41 ± 4	10 ± 7	4 ± 6	2 ± 5
fM	CCG	+	40 ± 2	7 ± 4	7 ± 8	2 ± 7
fM ^c	CCA	+	38 ± 4	4 ± 6	13 ± 10	0 ± 8
fM ^c	CCC	+	43 ± 5	13 ± 9	12 ± 13	6 ± 9
fM ^c	CCU	+	36 ± 5	30 ± 12	7 ± 16	9 ± 9
fM ^c	GGG	+	42 ± 4	10 ± 6	11 ± 11	6 ± 8
fMP	AUG	-	22 ± 1	48 ± 4	7 ± 3	6 ± 1
fM	AUA	-	17 ± 4	124 ± 30	28 ± 28	22 ± 8
fM ^b (PRE)	AUA	-	21 ± 10	98 ± 60	16 ± 34	2 ± 3
fM	CCG	-	17 ± 1	70 ± 6	5 ± 5	11 ± 2

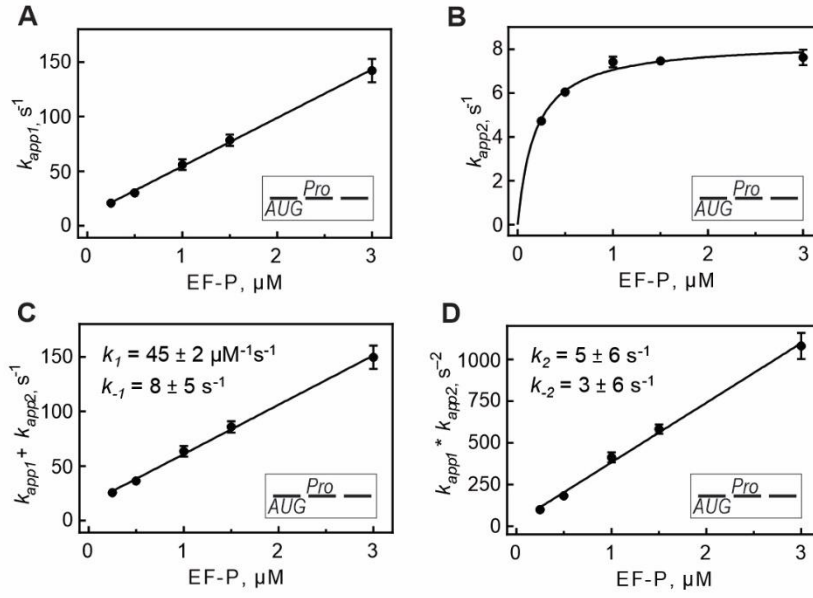
^a Unless stated otherwise, the values are averages and standard errors of fits from 3 independent biological replicates with 5 technical replicates each.

^b Shown is the average and standard error of fits from 2 independent biological replicates with 5 technical replicates each.

^c Shown is the average and standard error of fits from 5 technical replicates.



Supplementary Figure 1. Validation of the EF-P (D66C)-QSY35 activity. Functional activity of EF-P was estimated by its ability to accelerate peptide bond formation between f[³H]MetPro-tRNA^{Pro} in the P site (0.2 μ M) with puromycin (Pmn; 10 mM) in the A site of the ribosome. The experiment was carried out using a quench-flow apparatus, where reactants were rapidly mixed, incubated for the indicated times, then quenched, and the radioactive products analyzed by RP-HPLC (1). The reaction was measured using bL33-ATTO 488-labeled ribosome complexes in the absence (grey) or presence of EF-P-QSY35 (3 μ M; orange) or of unlabeled EF-P (3 μ M; black). The reaction rate was $0.2 \pm 0.1 \text{ s}^{-1}$ in the absence of EF-P, $4.2 \pm 0.8 \text{ s}^{-1}$ in the presence of native EF-P, and $9.4 \pm 0.8 \text{ s}^{-1}$ with EF-P-QSY35, indicating that fluorescence labeling did not impair the functional activity of the ribosome complex or EF-P.



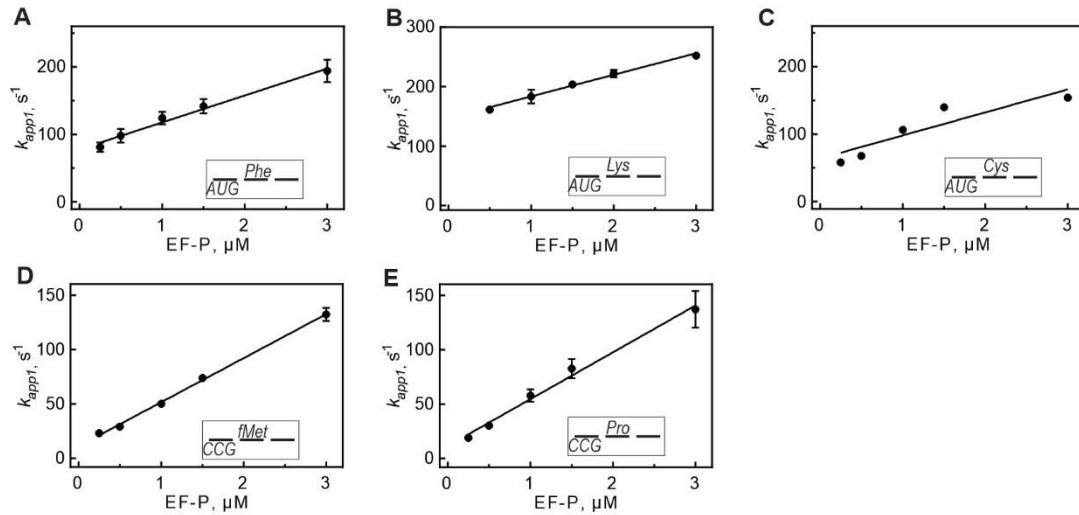
Supplementary Figure 2. Analysis of the concentration dependence of EF-P binding. Time courses shown in Fig. 1B were fitted to a model with two exponentials and a linear term (Materials and Methods). The estimated rate constants are summarized in Supplementary Table 1.

(A) Concentration dependence of the k_{app1} , comprising about 85% of the fluorescence change. The linear concentration dependence indicates that the fast step represents an association reaction. Error bars represent standard error of three biological replicates (N=3). The inset shows the codons and tRNAs in the E, P, and A sites (sites are indicated by dashes in the direction E–P–A from the left to the right). The mRNA codon is indicated below the line, tRNAs above the line.

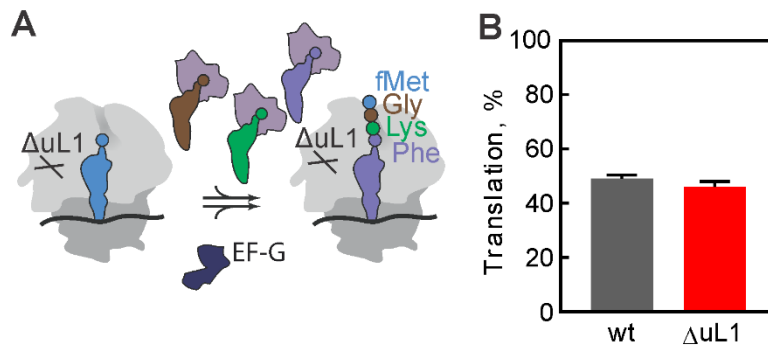
(B) Concentration dependence of k_{app2} . The hyperbolic fit (black line) is consistent with a model where k_{app2} represents the apparent rate of a rearrangement following the initial binding step.

(C) Concentration dependence of $k_{app1} + k_{app2}$. The slope of the plot yields the k_1 value; the Y-axis intercept equals to $k_{-1} + k_2 + k_{-2}$.

(D) Concentration dependence of $k_{app1} * k_{app2}$. The slope of the plot gives $k_1 * (k_2 + k_{-2})$, the Y-axis intercept $k_{-1} * k_2$. The values determined from linear fitting of the data in C and D allow to calculate elementary rate constants k_1 , k_{-1} , k_2 , and k_{-2} (Supplementary Table 1).



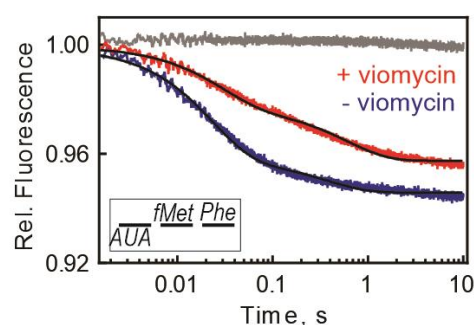
Supplementary Figure 3. Comparison of the concentration dependencies of the k_{app1} values of EF-P binding to various ribosome complexes derived from exponential fitting of data shown in Fig. 3. (A) fMetPhe-tRNA^{Phe} in the P site and AUG codon in the E site; (B) fMetLys-tRNA^{Lys} in the P site and AUG codon in the E site; (C) fMetCys-tRNA^{Cys} in the P site and AUG codon in the E site. fMetProPro-tRNA^{Pro} (D) or fMet-tRNA^{fMet} (E) in the P site, both with a CCG codon in the E site. (A, D, E) Error bars represent standard error of three biological replicates with 5 technical replicates each (N=3). (B, C) Error bars represent standard error of two biological replicates with 5 technical replicates each.



Supplementary Figure 4. Activity of the $\Delta uL1$ ribosomes in translation.

(A) Schematic of the experiment. Ribosomes (wt or $\Delta uL1$) were programmed with an mRNA coding for the fMet-Gly-Lys-Phe peptide. The efficiency of initiation was tested by radioactivity counting of f[³H]Met bound to the ribosome and was identical for both ribosomes. Then, ternary complexes specific for the Gly, [¹⁴C]Lys, and Phe codons were added together with EF-G and GTP and translation was carried out for 2 min at 37°C. After stopping the reaction, the products were separated by RP-HPLC and quantified by radioactive counting (2).

(B) Translational efficiency of the wt (gray) and $\Delta uL1$ (red) ribosomes expressed as the fraction of ribosomes carrying nascent chains of the expected lengths. Error bars represent standard error of three biological replicates (N=3).



Supplementary Figure 5. Binding of EF-P to rotated PRE state stabilized by the antibiotic viomycin (Vio). PRE complexes with tRNA^{fMet} in the P site and fMet-Phe-tRNA^{Phe} in the A site were prepared as described in the text for Fig. 2. EF-P was rapidly mixed with complexes in the absence of Vio (blue) or after the addition of Vio (0.2 mM) (red), which stabilizes the rotated PRE state of the ribosome (3). Apparent rate constants of the rapid binding step are: $43 \pm 1 \text{ s}^{-1}$ and $35 \pm 1 \text{ s}^{-1}$.

References

1. Doerfel, L.K., Wohlgemuth, I., Kothe, C., Peske, F., Urlaub, H. and Rodnina, M.V. (2013) EF-P is essential for rapid synthesis of proteins containing consecutive proline residues. *Science*, 339, 85-88.
2. Peng, B.Z., Bock, L.V., Belardinelli, R., Peske, F., Grubmuller, H. and Rodnina, M.V. (2019) Active role of elongation factor G in maintaining the mRNA reading frame during translation. *Sci. Adv.*, 5, eaax8030.
3. Ermolenko, D.N., Spiegel, P.C., Majumdar, Z.K., Hickerson, R.P., Clegg, R.M. and Noller, H.F. (2007) The antibiotic viomycin traps the ribosome in an intermediate state of translocation. *Nat Struct Mol Biol*, 14, 493-497.