A Kinetic Safety Gate Controlling the Delivery of Unnatural Amino Acids to the Ribosome

Joerg Mittelstaet, Andrey L Konevega, Marina V Rodnina

SUPPLEMENTARY FIGURES AND TABLES

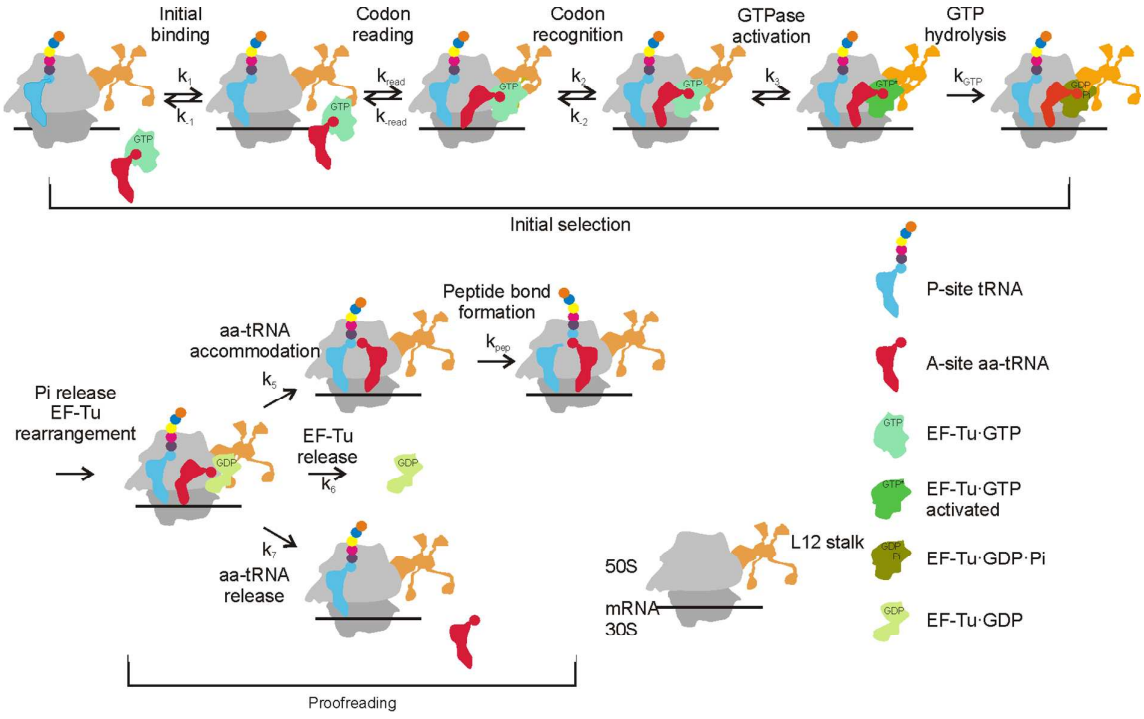

Figure S1. Schematic of the sequence of events and tRNA selection steps during mRNA decoding in the A site of the ribosome.

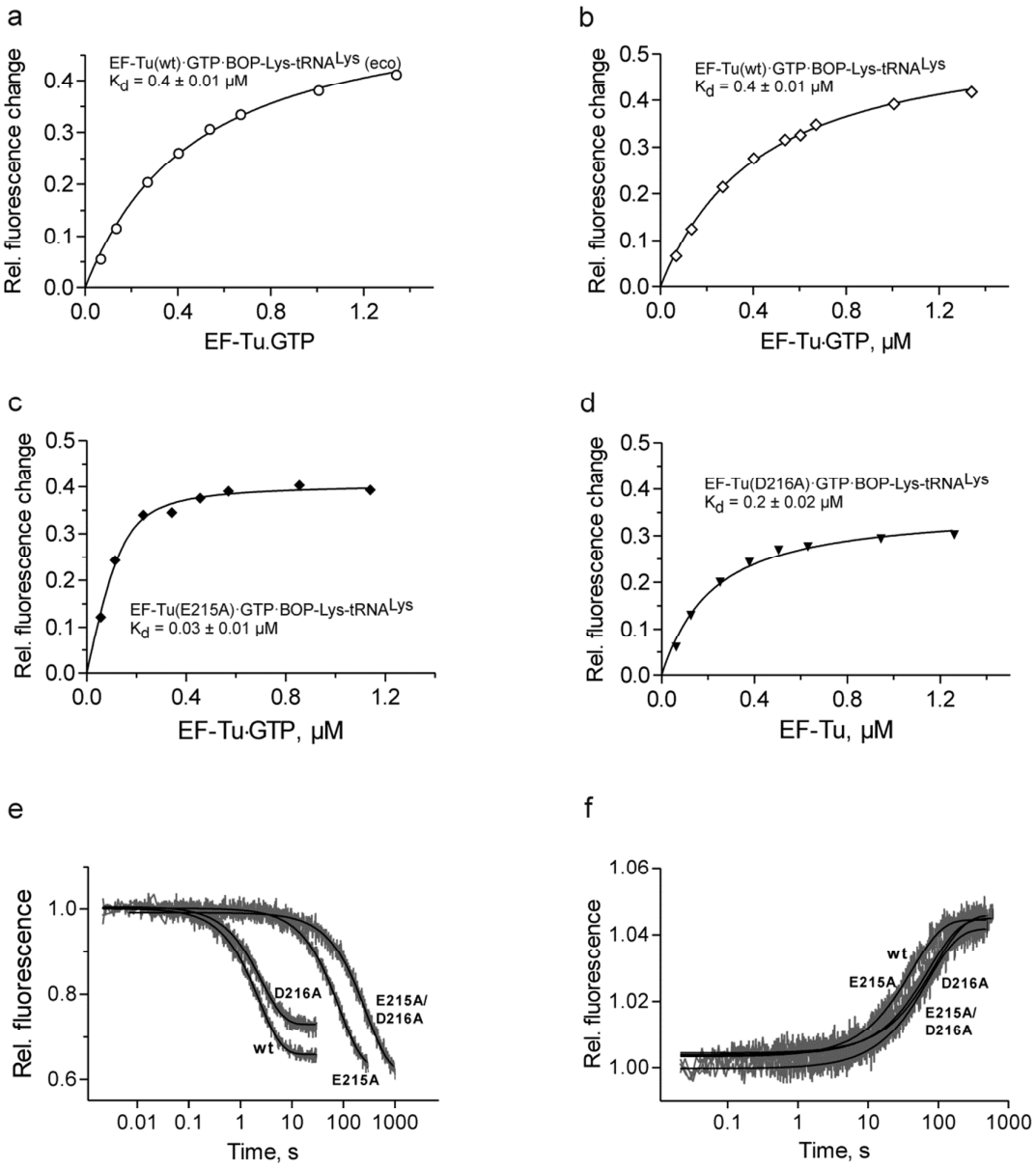

Figure S2. Evaluation of K_d and k_{off} values. (a-d) Examples of concentration dependences of the fluorescence change upon binding of EF-Tu (wt or mutants, as indicated) to BOP-Lys-tRNA^{Lys}. (e) Chase of BOP-Lys-tRNA^{Lys} (0.05 μM) from EF-Tu–GTP by the addition of excess unlabeled aa-tRNA (40 μM). (f) Chase of Lys-tRNA^{Lys}(Flu) (0.05 μM) from EF-Tu–GTP by excess unlabeled aa-tRNA (40 μM).

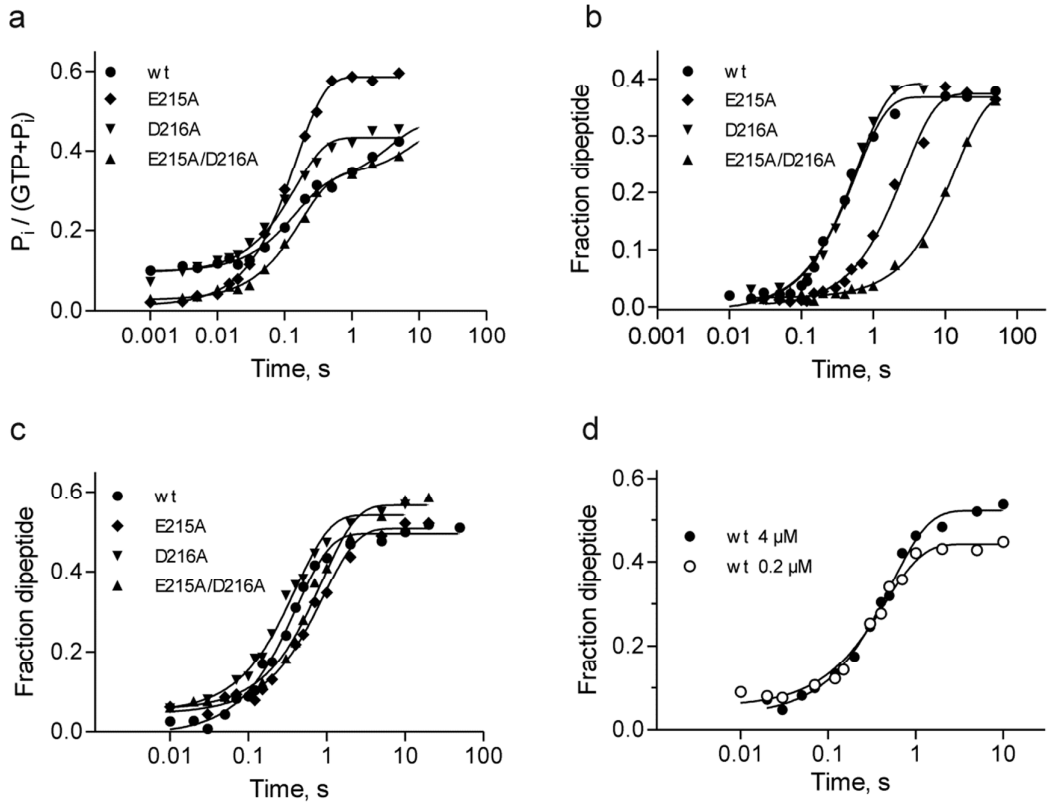

Figure S3. Time courses of GTP hydrolysis (a) and peptide bond formation (b,c,d) with wt and mutant EF-Tu. (a) GTP hydrolysis upon binding of the ternary complex EF-Tu–[γ-^{32}P]GTP–BOP-Lys-tRNA^{Lys} (0.1 µM) to initiation complexes with a cognate AAA codon in the A site (0.3 µM). BOP-Lys-tRNA^{Lys} was present in large excess (1 µM) to ensure complex formation also for the EF-Tu mutant forming the weakest complex. Thin lines represent single and double-exponential fits resulting in rates of 7.2 ± 1.2 s^{-1} (rapid phase) for EF-Tu(wt) (●), 6.8 ± 0.2 s^{-1} for EF-Tu(E215A) (◆), 6.8 ± 0.7 s^{-1} for EF-Tu(D216A) (▼), and 5.7 ± 0.4 s^{-1} (rapid phase) for EF-Tu(E215A/D216A) (▲). (b) Peptide bond formation with BOP-Lys-tRNA^{Lys}. The complex of EF-Tu–GTP (4 µM) and BOP-Lys-tRNA^{Lys} (0.1 µM) was rapidly mixed with initiation complexes (0.3 µM). Thin lines represent single-exponential fits resulting in rates of 1.7 ± 0.1 s^{-1} for EF-Tu(wt) (●), 0.36 ± 0.03 s^{-1} for EF-Tu(E215A) (◆), 1.6 ± 0.1 s^{-1} for EF-Tu(D216A) (▼), and 0.07 ± 0.01 s^{-1} for EF-Tu(E215A/D216A) (▲). (c) Peptide bond formation with Lys-tRNA^{Lys}. The complex of EF-Tu–GTP (4 µM) and Lys-tRNA^{Lys} (0.1 µM) was rapidly mixed with initiation complexes (0.3 µM). Single-exponential fits resulted in rates of 2.4 ± 0.1 s^{-1} for EF-Tu(wt) (●),1.1 ± 0.1 s^{-1} for EF-Tu(E215A) (◆), 2.4 ± 0.2 s^{-1} for EF-Tu(D216A) (▼), and 1.1 ± 0.1 s^{-1} for EF-Tu(E215A/D216A) (▲). (d) Peptide bond formation with Lys-tRNA^{Lys}(Flu) (0.1 µM) in presence of high (4 µM) and low (0.2 µM) concentration of EF-Tu(wt)–GTP upon mixing with initiation complexes (0.3 µM). The rates are 1.9 ± 0.1 s^{-1} and 2.2 ± 0.2 s^{-1}, at 4 µM and 0.2 µM EF-Tu, respectively.

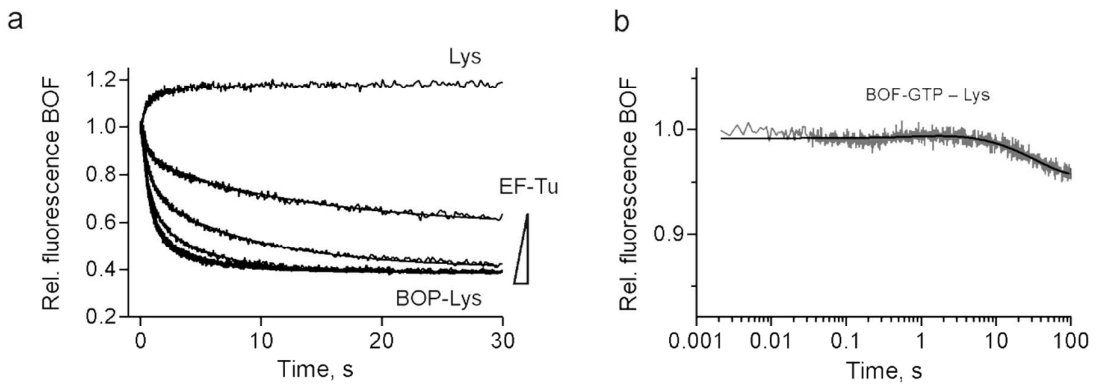

Figure S4. (a) Accommodation of aa-tRNA (0.1 μM) on the ribosome carrying BOF-Met-tRNA^{fMet} in the P site (0.05 μM) at increasing EF-Tu concentrations. Traces show the decrease of donor (BOF) fluorescence upon binding of BOP-Lys-tRNA^{Lys} at different EF-Tu–GTP concentrations (right to left: 0.2, 0.4, 0.8, 1.6, 2.8, and 4.0 μM). The binding of the ternary complex with unlabeled Lys-tRNA^{Lys} (at 4.0 μM EF-Tu-GTP) is shown for comparison (top-most trace). (b) BOF fluorescence change upon interaction of EF-Tu–BOF-GTP–Lys-tRNA^{Lys} with the ribosome. The dissociation rate of BOF-GDP from EF-Tu(E215A/D216A) as measured upon addition of excess non-labeled GDP[1] was 0.02 s^{-1}, which is identical to the observed rate of fluorescence decrease (0.02 s^{-1}) in the shown time course.

Reference:

(1) Gromadski, K. B.; Wieden, H. J.; Rodnina, M. V. *Biochemistry* **2002**, *41*, 162.

Supplementary Table 1. Binding of BOP-Lys-tRNA^{Lys} to EF-Tu–GTP and rate of peptide bond formation on the ribosome

EF-Tu	K_d, μM [a]	k_{on}, μM^{-1} s^{-1} [c]	k_{off}, s^{-1} [d]	$k_{off(chase)}$, s^{-1} [e]	k_{pep}, s^{-1} [f]
wt	0.43 ± 0.02 [b]	1.0	0.48	0.43	1.7 ± 0.1
wt	0.43 ± 0.05 [b]	1.5[a]	0.69[a]	-	-
D216A	0.16 ± 0.03 [b]	0.8[a]	0.69[a]	0.39	1.6 ± 0.1
E215A	0.02 ± 0.01[b]	2.8[a]	0.05[a]	0.01	0.36 ± 0.03
E215A/D216A	0.004 ± 0.002 [b]	1.7[a]	~0[a]	0.004	0.072 ± 0.004

[a] K_d values are averages of values determined form the amplitude dependence of the titration (Supplementary Figures 2a-d) and from k_{off}/k_{on}; in the latter case, the k_{off} value from the chase experiments was taken due to its higher precision.

[b] tRNA^{Lys} transcript was used for the titration experiment

[c] Standard deviation was <2% of k_{on} values.

[d] Determined from the Y-axis intercept of linear concentration dependence of k_{app} (Figure 2c). Standard deviation of values was 2-20%.

[e] From the tRNA dissociation experiments (Supplementary Figure 2e). Native tRNA^{Lys} was used. Standard deviation of values was <2%.

[f] determined with EF-Tu–GTP (4 μM), BOP-Lys-tRNA^{Lys} (0.1 μM), and 70S ribosomes with fMet-tRNA^{fMet} in the P site and the AAA codon in A site (0.3 μM).

Supplementary Table 2. Binding of Lys-tRNA^{Lys} to EF-Tu·GTP and rate of peptide bond formation on the ribosome

EF-Tu	K_d, μM [a]	$k_{off(chase)}$, s^{-1} [a, b]	k_{pep}, s^{-1} [c]
wt	0.009 ± 0.003	0.013	2.4 ± 0.1
D216A	-	0.0140	2.4 ± 0.2
E215A	-	0.024	1.1 ± 0.1
E215A/D216A	-	0.011	1.1 ± 0.1

[a] The K_d value is an average of the K_d determined from the amplitude dependence of the titration and from k_{off}/k_{on}. The k_{on} and k_{off} values for Lys-tRNA^{Lys}(Flu) binding to EF-Tu(wt) determined from the concentration dependence of the k_{app} value (Figure 2c) were 1.27 ± 0.02 $\mu M^{-1}s^{-1}$ and ~0 s^{-1}, respectively.

[b] Standard deviation of values was <2%

[c] determined with EF-Tu–GTP (4 μM), Lys-tRNA^{Lys} (0.1 μM), and 70S ribosomes with fMet-tRNA^{fMet} in the P site and the AAA codon in A site (0.3 μM).