Supporting Information

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SI Text

SI Methods. *Data evaluation.* When a ternary complex, consisting of EF-Tu, aa-tRNA, and GTP, reacts with an initiated 70S ribosome, a series of events take place (1). As shown in Fig. 3*A* the time evolution of peptide bond formation in the single-round experiments used here, has a sigmoidal look, due to two or more chemical steps with kinetics in the same time scale. As we have shown earlier, it is correct to evaluate the results from this type of experiment using mean-time calculations (2). By simply integrating the area, up to one, above a normalized time course of e.g., dipeptide formation, the mean-time of the overall reaction is achieved, i.e.:

$$\tau_{\mathrm{dip}} = \int_0^{\infty} \left(1 - \frac{\mathrm{dip}(t)}{\mathrm{dip}(\infty)}\right) \mathrm{dt}. \qquad \textbf{[S1]}$$

Similarly, the average time for GTP hydrolysis, τ_{GTP}, is:

$$\tau_{\mathrm{GTP}} = \int_0^{\infty} \left(1 - \frac{\mathrm{GDP}(t)}{\mathrm{GDP}(\infty)}\right) \mathrm{dt}. \qquad \textbf{[S2]}$$

For the simplified scheme in Fig. 1, the difference between τ_{dip} and τ_{GTP}, τ_{pep}, can be expressed from the individual rate constants as:

$$\tau_{\mathrm{pep}} = \tau_{\mathrm{dip}} - \tau_{\mathrm{GTP}} = \frac{1}{k_{\mathrm{tu}}} + \frac{1}{k_{\mathrm{ac}}} + \frac{1}{k_{\mathrm{pt}}}. \qquad \textbf{[S3]}$$

If release of EF-Tu·GDP occurs in parallel with accommodation (1), then the term $1/k_{\mathrm{tu}}$ is excluded from the right hand side of Eq. **S3**. In our experiments we were interested in the steps subsequent to hydrolysis of EF-Tu-bound GTP. Accordingly we monitored the time evolution of GTP hydrolysis *and* dipeptide formation in the very same experiment (Fig. 3*A*). The time course of GTP hydrolysis, including binding of T_3 to 70S ribosomes, codon-anticodon recognition, GTPase activation, and GTP hydrolysis (1), could in all cases be excellently fitted to a single exponential expression. With *bg* a constant background, the mean-time for GTP hydrolysis, τ_{GTP}, was estimated from:

$$\mathrm{GTP}(t) = P\left(1 - e^{-\frac{t}{\tau_{\mathrm{GTP}}}}\right) + bg. \qquad \textbf{[S4]}$$

The standard deviation, $\sigma_{\tau_{\mathrm{GTP}}}$, of τ_{GTP} was provided by the fitting program [Levenberg-Marquardt algorithm fit using Origin (OriginLab Corp.)]. For dipeptide formation, the curves were fitted to a two-step reaction model containing the mean-time for dipeptide formation, τ_{dip}, as one of the parameters:

$$\mathrm{dip}(t) = \frac{R_0}{k_1 - \frac{1}{\tau_{\mathrm{dip}} - 1/k_1}} \left(k_1 \cdot \left(1 - e^{\left(-\frac{t}{\tau_{dip} - 1/k_1}\right)}\right) - \frac{1}{\tau_{\mathrm{dip}} - 1/k_1}\left(1 - e^{-k_1 \cdot t}\right)\right) + bg. \qquad \textbf{[S5]}$$

The use of τ_{dip} rather than the second rate constant in the model allowed us to obtain an estimate of the standard deviation, $\sigma_{\tau_{\mathrm{dip}}}$, of τ_{dip} directly from the fit as in the case of $\sigma_{\tau_{\mathrm{GTP}}}$. The mean-time for all the steps *subsequent* to GTP hydrolysis up to peptidyl transfer, τ_{pep}, was estimated as:

$$\tau_{\mathrm{pep}} = \tau_{\mathrm{dip}} - \tau_{\mathrm{GTP}}. \qquad \textbf{[S6]}$$

The standard deviation, $\sigma_{\tau_{\mathrm{pep}}}$, of τ_{pep} was estimated as:

$$\sigma_{\tau_{\mathrm{pep}}} = \sqrt{\sigma^2_{\tau_{\mathrm{dip}}} + \sigma^2_{\tau_{\mathrm{GTP}}}}. \qquad \textbf{[S7]}$$

The corresponding reaction rate, k_{pep}, and its standard deviation, $\sigma_{k_{\mathrm{pep}}}$, were estimated as:

$$k_{\mathrm{pep}} = \frac{1}{\tau_{\mathrm{pep}}}, \qquad \sigma_{k_{\mathrm{pep}}} = \frac{\sigma_{\tau_{\mathrm{pep}}}}{\tau^2_{\mathrm{pep}}}. \qquad \textbf{[S8]}$$

All experiments were repeated at least twice. Maximum likelihood estimates of average k_{pep} values were obtained from single experiment variances ($w_i = 1/\sigma^2_{\mathrm{kpepi}}$) by minimizing the χ^2 function:

$$\chi^2 = \sum_{i=1}^{n} w_i \cdot (k_{\mathrm{pep}(i)} - k_{\mathrm{pep}})^2, \qquad \textbf{[S9]}$$

with respect to k_{pep}. From this expression we obtained:

$$k_{\mathrm{pep}} = \frac{\sum_{i=1}^{n} w_i \cdot k_{\mathrm{pep}(i)}}{\sum_{i=1}^{n} w_i}, \qquad \sigma_{\mathrm{kpep}} = \frac{1}{\sqrt{\sum_{i=1}^{n} w_i}}. \qquad \textbf{[S10]}$$

According to the present results with peptidyl transfer between native tRNAs, only one reacting group is being protonated when pH is changed near physiological pH, i.e., the α-ammonium proton on the aminoacyl-tRNA. The pH dependence of peptidyl transfer, k_{pt} in Fig. 1, is therefore (3):

$$k_{\mathrm{pt}} = \frac{k^{\mathrm{max}}_{\mathrm{pt}}}{1 + 10^{(pK^{\mathrm{ribo}}_a - \mathrm{pH})}}. \qquad \textbf{[S11]}$$

Here, the rate at complete protonation is assumed to be zero, in line with the current understanding of aminolysis (4, 5). From our experiments, we calculate the compounded rate constant, k_{pep}, of all steps subsequent to GTP hydrolysis as the inverse of the time τ_{pep} (See Fig. 3*A* and Eq. **S8** above). The parameters $k^{\mathrm{max}}_{\mathrm{pep}}$ and pK^{obs}_a, in Table 1, were estimated by fitting the experimentally observed pH variation of k_{pep} to the function:

$$k_{\mathrm{pep}} = \frac{k^{\mathrm{max}}_{\mathrm{pep}}}{1 + 10^{(pK^{\mathrm{obs}}_a - \mathrm{pH})}}. \qquad \textbf{[S12]}$$

The observed pK^{obs}_a value is related to the pK_a value of the α-amino group of a ribosome bound aa-tRNA, pK^{ribo}_a, and the "downshift term" $\log_{10}(k^{\mathrm{max}}_{\mathrm{pep}}/k^{\mathrm{max}}_{\mathrm{pt}})$ through:

$$\mathrm{pK}^{\mathrm{obs}}_a = pK^{\mathrm{ribo}}_a + \log_{10}\left(\frac{k^{\mathrm{max}}_{\mathrm{pep}}}{k^{\mathrm{max}}_{\mathrm{pt}}}\right). \qquad \textbf{[S13]}$$

The resolution of our kinetic experiments allows precise estimates of the average times τ_{dip}, τ_{GTP}, and τ_{pep}, but not the downshift ratio $k^{\mathrm{max}}_{\mathrm{pep}}/k^{\mathrm{max}}_{\mathrm{pt}}$ in Eq. **S13** (see *Discussion* in the main text). When pK^{ribo}_a is equal to the pK_a-value of aminoacyl-tRNAs or their analogues in bulk water, pK^{aq}_a, $k^{\mathrm{max}}_{\mathrm{pt}}$ can be calculated from observed data and Eq. **S13** as:

$$k^{\mathrm{max}}_{\mathrm{pt}} = k^{\mathrm{max}}_{\mathrm{pep}} \cdot 10^{pK^{\mathrm{ribo}}_a - pK^{\mathrm{obs}}_a}. \qquad \textbf{[S14]}$$

The compounded rate constant of the steps *between* GTP hydrolysis and the chemistry of peptide bond formation can be calculated as:

$$\frac{1}{1/k_{\mathrm{pep}}^{\max} - 1/k_{\mathrm{pt}}^{\max}}. \qquad \textbf{[S15]}$$

MD-simulated pK_a-values of A-site bound aminoacyl-tRNAs. In order to examine the relation between the pK_a^{obs}-values (Table 1, column 3) and the physical process of ionizing the α-amino group in the ribosomal A site we carried out molecular dynamics (MD) simulations of the CCA end of the six different aa-tRNAs in both the neutral and protonated form in bulk water as well as in the ribosome with a dipeptide bound to the P-site tRNA. The systems including solvent and ions were prepared using essentially the same protocols as described previously (6, 7) and comprised all residues with atoms within 20 Å of the P-site carboxyl carbon. Atoms within the system boundary were fully mobile while atoms outside the 20 Å radius were restrained to their initial positions with a 100 kcal/(mol·Å^2) harmonic force constant. No nonbonded interactions outside or across the boundary was calculated and water molecules close to the boundary were restrained to reproduce the correct density and polarization (8). All nonbonded interactions were calculated for the α-amine while for other interactions a multipole expansion treatment (9) of long-range electrostatics (beyond 10 Å) was employed. The different systems were heated from 1 K to 300 K in a step wise manner with initial random velocities taken from a Maxwell-Boltzmann distribution and the temperature was kept constant by coupling to an external heat bath. The MD simulations used a time step of 1 fs and the systems were equilibrated at 300 K for at least 300 ps before data collection. Data were collected every 0.1 ps for all systems during subsequent MD simulations of 4 ns each. The same set of simulations were performed for each of the six aa-tRNAs with the α-amine protonated. Finally, an identical simulation scheme was repeated for each of the six amino acid esters in a 20 Å sphere of water, with the α-amine both neutral and protonated.

The pK_a-shifts of the ribosome bound aa-tRNAs, relative to their unperturbed values in aqueous solution, are given by the thermodynamic cycle corresponding to moving the neutral and protonated aa-tRNA species from water to the ribosomal A-site (Scheme S1)

Here, ΔG^{aq} and ΔG^{ribo} are the differences in standard free energy between the protonated and deprotonated forms of the α-amino groups of aminoacyl-tRNAs in bulk water (*aq*) and ribosomal A site (*ribo*), respectively. It follows directly from the definitions of pK_a-values for ribosome bound, pK_a^{ribo}, and free, pK_a^{aq}, aminoacyl-tRNAs that:

$$\begin{aligned}\mathrm{RT}\log 10(pK_a^{\mathrm{ribo}} - pK_a^{\mathrm{aq}}) &= \mathrm{RT}\log 10\Delta pK_a^{\mathrm{calc}} \\ &= -(\Delta G^{\mathrm{ribo}} - \Delta G^{\mathrm{aq}}) = -\Delta\Delta G.\end{aligned} \qquad \textbf{[S16]}$$

The absolute standard free energy differences ΔG^{ribo} and ΔG^{aq} are difficult to calculate because they in principle depend on the dielectric properties of the entire solvated ribosome. However, the relative free energies between different aa-tRNAs are easier to obtain as long-range electrostatic effects can then be expected to cancel. Here, we estimate these free energy differences by the semiempirical linear interaction (LIE) method (10) which relates the average polar and nonpolar interactions between the ionizing amino acid ester and its surroundings (i.e., the potential energy differences $\Delta\langle U_{\mathrm{el}}\rangle^{\mathrm{ribo}}$, $\Delta\langle U_{\mathrm{vdw}}\rangle^{\mathrm{ribo}}$, $\Delta\langle U_{\mathrm{el}}\rangle^{aq}$ and $\Delta\langle U_{\mathrm{vdw}}\rangle^{\mathrm{aq}}$) to the corresponding free energy contributions. This method gives a linear relation between ΔpK_a and the average energies are obtained, where the slope, $\Delta\Delta pK_a^{\mathrm{calc}}$, is given by:

$$-1.34\Delta\Delta pK_a^{\mathrm{calc}} = 0.18\Delta\Delta\langle U_{\mathrm{vdw}}\rangle + \beta\Delta\Delta\langle U_{\mathrm{el}}\rangle. \qquad \textbf{[S17]}$$

Here, $\Delta\Delta\langle U_{\mathrm{el}}\rangle = \Delta\langle U_{\mathrm{el}}\rangle_{\mathrm{ribo}} - \Delta\langle U_{\mathrm{el}}\rangle_{\mathrm{aq}}$, $\Delta\Delta\langle U_{\mathrm{vdw}}\rangle = \Delta\langle U_{\mathrm{vdw}}\rangle_{\mathrm{ribo}} - \Delta\langle U_{\mathrm{vdw}}\rangle_{\mathrm{aq}}$, β is an electrostatic scaling constant for the surroundings of the peptidyl transferase center (PTC) and 0.18 is a standard scaling factor relating van der Waals interactions ($\Delta\langle U_{\mathrm{vdw}}\rangle$) to nonpolar free energy contributions (11), while 1.34 is RTlog10 (at 20 °C). MD simulations were used to calculate $\Delta\Delta\langle U_{\mathrm{vdw}}\rangle$ and $\Delta\Delta\langle U_{\mathrm{el}}\rangle$ for all six aminoacyl-tRNAs.

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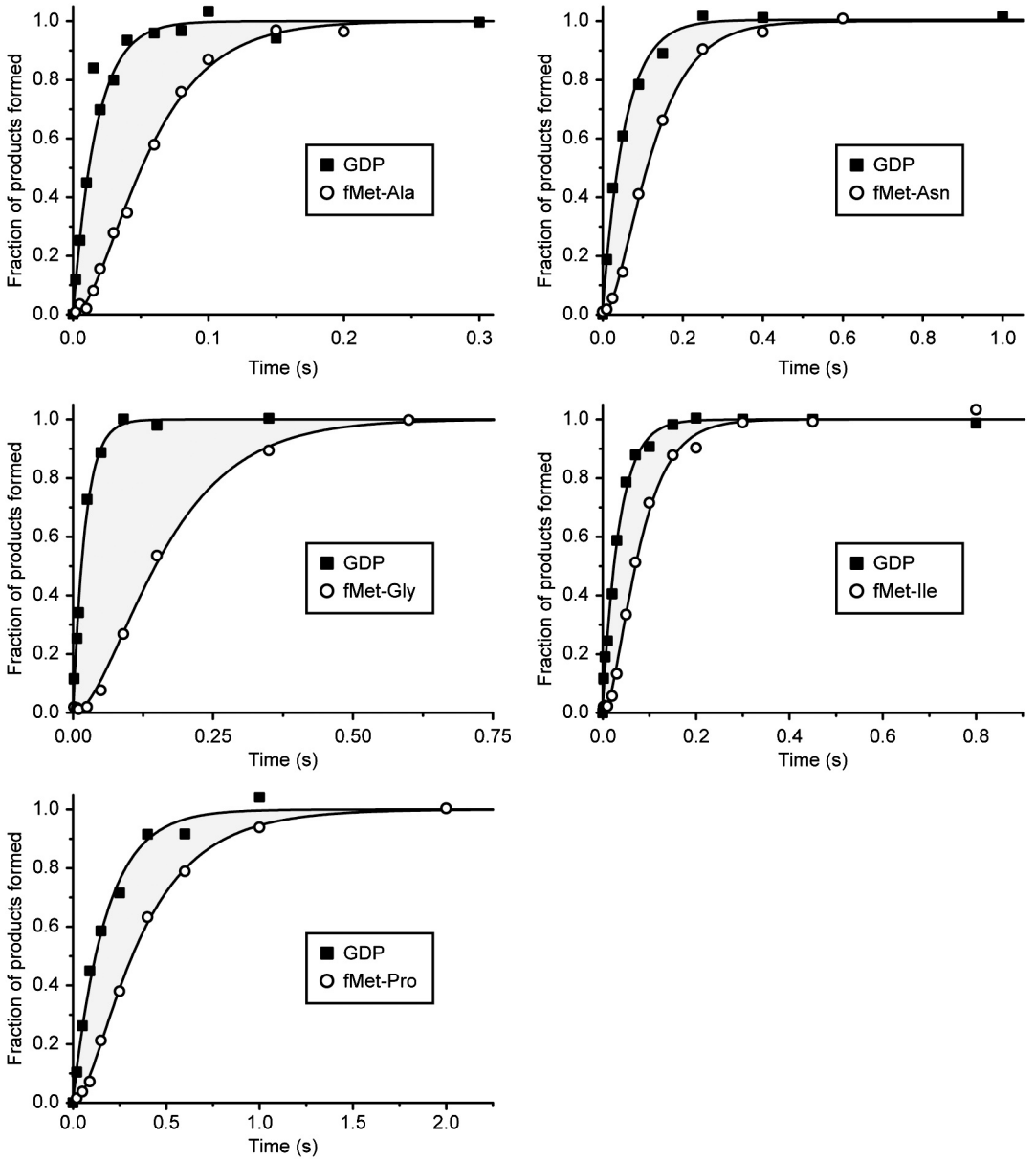


Fig. S1. The normalized amount of GDP and dipeptide formed as a function of time when EF-Tu·aa-tRNA·[^{3}H]GTP ternary complexes (aa-tRNAs as indicated in the figure) react at ~pH 7.5 and 20 °C with f[^{35}S]Met-tRNA^{fMet} initiated 70S ribosomes displaying GCA (Ala), AAC (Asn), GGC (Gly), AUC (Ile), or CCC (Pro) codon in A-site. The shaded areas represent the mean-time, τ_{pep}, for all reaction steps subsequent to GTP hydrolysis up to and including peptidyl transfer.

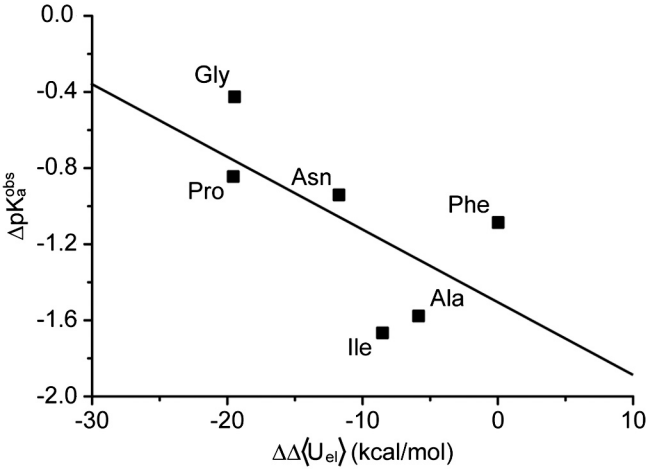
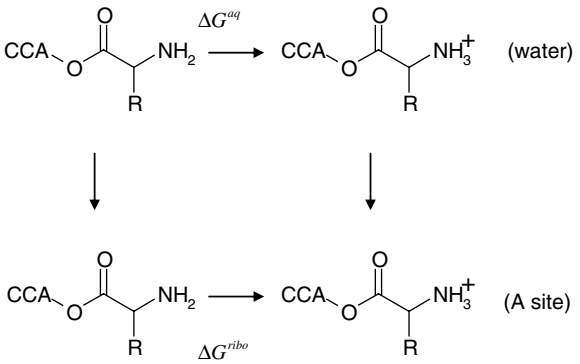


Fig. S2. MD-simulated electrostatic potential energy differences, $\Delta\Delta\langle U_{el}\rangle$, for the neutral and ionized form of the α-amino group of an A-site bound or free aa-tRNA, plotted vs. experimentally observed pK_a shifts, $\Delta pK_a^{obs} = pK_a^{obs} - pK_a^{aq}$ (Table 1). Phe-tRNA^{Phe} is set as standard (=0 kcal/mol) for the relative values of $\Delta\Delta\langle U_{el}\rangle$.



Scheme S1.