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Table S1 | Activation parameters of uncatalyzed and catalyzed peptide bond formation (25°C).

Amine	Ester	Activation parameters (kcal/mol)		
		$\Delta G^{\neq}$	$\Delta H^{\neq}$	$T\Delta S^{\neq}$
Uncatalyzed				
Tris[a]	fMet-tRNA^{fMet}	22.7	16.2	-6.5
Catalyzed by 70S ribosomes				
Pmn[b]	fMetPhe-tRNA^{Phe}	14.0	16.0	2.0
Pmn[c]	fMetPhe-tRNA^{Phe}	16.5	17.2	0.7
Pmn[d]	fMet-tRNA^{fMet}	14.6	14.4	-0.2

[a] Data from ref.[1]

[b] Measured at k_{cat}/K_M conditions of puromycin (Pmn) comparable to the second-order reaction of model substrates in solution[2].

[c] Measured at saturating concentration of Pmn (k_{cat} conditions)[2].

[d] At k_{cat}/K_M conditions, present work; standard deviation of values is ≤3%.

1 Beringer, M. & Rodnina, M. V. The ribosomal peptidyl transferase. *Mol. Cell* **26**, 311-321 (2007).

2 Sievers, A., Beringer, M., Rodnina, M. V. & Wolfenden, R. The ribosome as an entropy trap. *Proc. Natl. Acad. Sci. USA* **101**, 7897-7901 (2004).

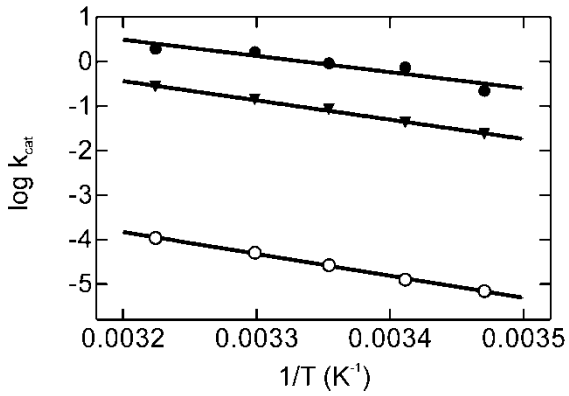

Figure S1 | Temperature dependence of fMet-tRNA^{fMet} hydrolysis. Rate constants (k_{cat}) of the ribosome-RF2-catalyzed fMet-tRNA^{fMet} hydrolysis with methylated RF2 (●), with unmethylated RF2 (▼), and the uncatalyzed reaction (○) were measured in buffer A at different temperatures. Values of log k_{cat} (s^{-1}) are plotted against the inverse absolute temperature; standard deviations of values are smaller than symbols. From the slopes of the linear plots, activation energies (E_a) were determined. Activation parameters derived from E_a measured at 25°C are summarized in Table 1.

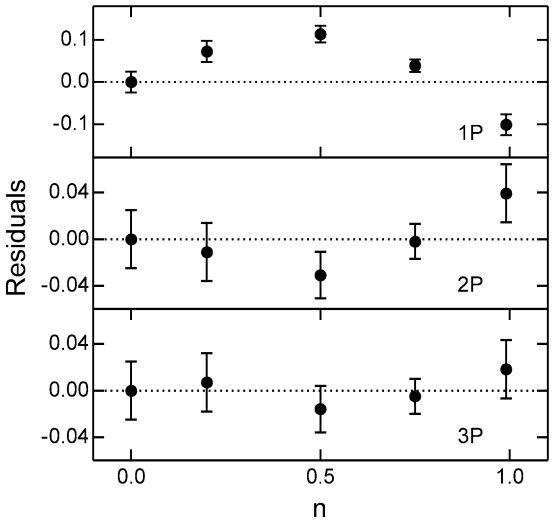

Figure S2 | Residuals of proton inventory fits of peptide bond formation. Plotted are the deviations of the measured points of Fig. 3a (circles) from fits (dotted lines) assuming the concerted movement of one (1P), two (2P) or three (3P) protons in the transition state. Absolute sum of squares was 0.0026 for the 2P model, compared to 0.00065 for the 3P model, chi-squares 0.0172 and 0.0034, respectively.