Supplementary Data for

Entropic contribution of elongation factor P to proline positioning at the catalytic center of the ribosome

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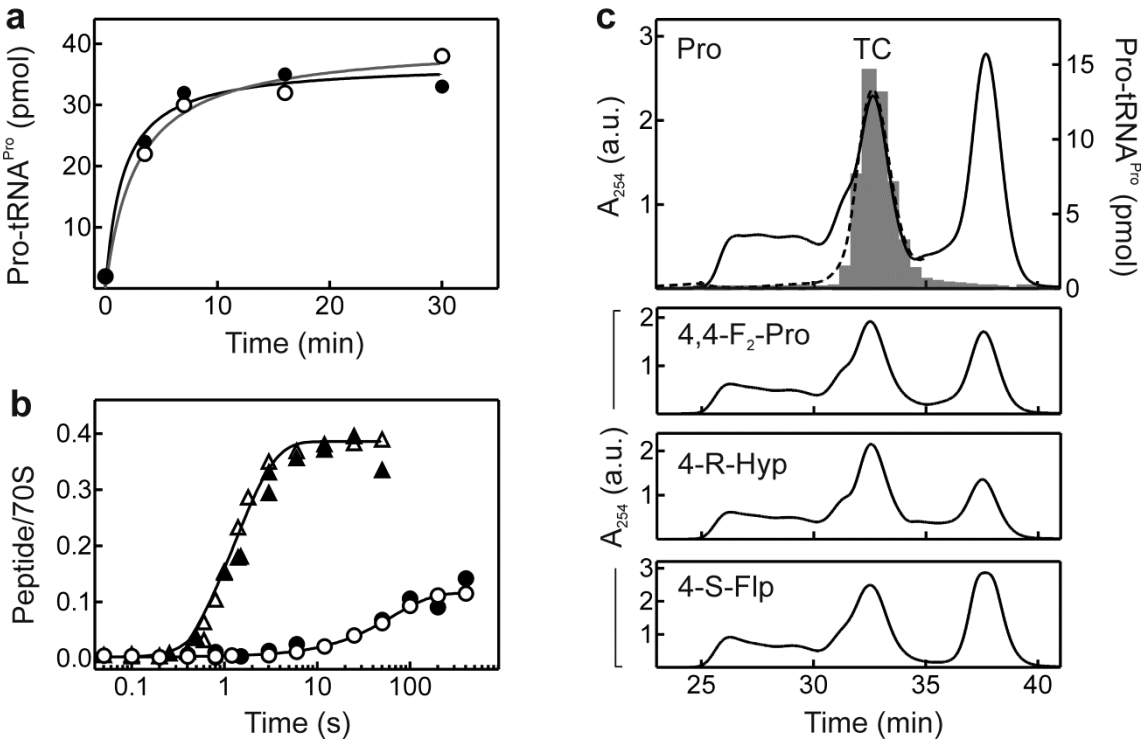

Figure S1: Functional characterization of Pro*-tRNA^{Pro}. (a) Aminoacylation of tRNA^{Pro} obtained from cells (closed circles) and by T7 RNA-polymerase transcription (open circles) with [^{14}C]Pro by purified Pro-RS. (b) fMPPG with native tRNA^{Pro} (open symbols) and tRNA^{Pro} transcript (closed symbols) in the absence (circles) or presence of EF-P (triangles). (c) Isolation of the TC by SEC. The upper panel shows purification of [^{14}C]Pro-tRNA^{Pro} ternary complex (with EF-Tu and GTP). The middle peak corresponds to the TC, as shown by radioactively labeled aa-tRNA. The first peak corresponds to Pro-RS (the dashed profile was obtained with purified aa-tRNA) and the third peak contains uncharged tRNA and EF-Tu. The lower panels show the profile for TCs with X-tRNA^{Pro} with X= 4,4-F_2-Pro, 4-R-Hyp and 4-S-Flp as representatives for Pro analogs. The ratio tRNA/EF-Tu is kept constant, such that the TC-forming efficiency can be estimated from the ratio of peak2/peak3.

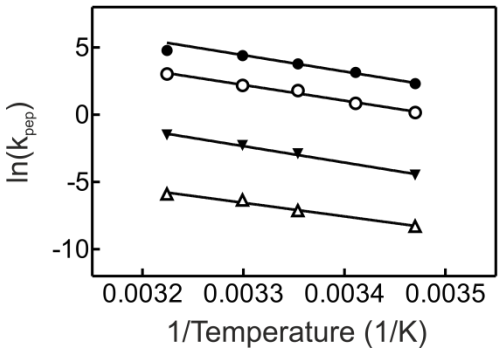

Figure S2: Arrhenius plots of fMP*-Pmn formation with and without EF-P.
The Pmn reaction with fMet-S-Flp-tRNA^{Pro} (triangles) and fMet-R-Flp-tRNA^{Pro} (circles), without EF-P (open symbols) or with EF-P (closed symbols). Shown are average values and SD from up to four replicates. Because the rate of the Pmn reaction with fMet-R-Flp-tRNA^{Pro} in the presence of EF-P at 37°C was too rapid for the quench-flow apparatus, the value could not be determined with precision and was therefore excluded from the fitting.

Table S1: Equilibrium and kinetic parameters of amide rotation for Pro and analogs.

Pro*	$-\Delta G_{tc}$[a], kcal/mol	Ref.	$-\Delta G_{tc}$[b], kcal/mol	Ref.	k_{ct}(Pro*)/ k_{ct}(Pro)	Ref.
Pro	0.90 ± 0.03[c]	[1]	0.92 ± 0.02	[2]	= 1	-
Aze	0.82 ± 0.04[d]	[3]	-	-	15[d,e] (19[e,f])	[3]
Pip	1.13 ± 0.05[d]	[3]	-	-	49 [d,e] (54 [e,f])	[3]
4-S-Flp	0.54 ± 0.02[c]	[1]	0.54 ± 0.02	[2]	1.2[c,e]	[2]
4-R-Flp	1.13 ± 0.04[c]	[1]	1.12 ± 0.12	[2]	2.1[c,e]	[2]
4,4-F_2-Pro	0.76 ± 0.03[c]	[4]	0.70 ± 0.06	[2]	5.2[c,e]	[2]
4-S-Hyp [g]	0.49 ± 0.02[c]		0.54 ± 0.16		0.53[c,e]	
4-R-Hyp	1.07 ± 0.04[c]	[1]	1.08 ± 0.04	[5]	0.67[c,e] (1.1[e,h])	[5] ([6])
cis-MePro [g]	1.04 ± 0.04[c]		-	-	6.6[e,i] (0.9 [e,f])	[j]
trans-MePro [g]	1.40 ± 0.05[c]		-	-	16[e,i] (16 [e,f])	[j]
4-S-Mep	1.19 ± 0.04[c]	[1]	-	-	0.67[h,k]	[7]
4-R-Mep	0.77 ± 0.03[c]	[1]	-	-	0.09 [h,k]	[7]
3,4-Dhp [g]	1.00 ± 0.03[c]		0.98 ± 0.08		0.30[c,e] (2.1[h,k])	([8])
Ala	3.04 ± 0.05[l]	[9]	-	-	-	-
Phe	3.03 ± 0.01[l]	[9]	-	-	-	-
Val	3.24 ± 0.11[l]	[9]	-	-	-	-

Values were determined in Ac-Pro*-X; [a] calculated as $\Delta G = -RT\ln K$ for 298 K; [b] calculated as $\Delta G = \Delta H - T\Delta S$; [c] X = OCH_3; [d] X = 4-nitroanilide; [e] experimental value; [f] X = O^-; [g] values determined in this work; [h] X = $NHCH_3$; [i] within Ac-GG-Pro*-GG-NH_2 peptides; [j] to be published elsewhere; [k] theoretical value; [l] X = OH

Ac-4-S-Hyp-OCH_3 ^{1}H NMR (D_2O, 700 MHz): 4.73 (dd, J = 7.4, 3.2 Hz, 1H, α-CH, cis), 4.56 (dd, J = 9.6, 2.6 Hz, 1H, α-CH, trans), 4.47 (m, 1H, γ-CH, trans), 4.43 (m, 1H, γ-CH, cis), 3.74 (dd, J = 11.7, 4.5 Hz, 1H, δ-CH1, trans), 3.71 (s, 3H, CH3O, cis), 3.67 (s, 3H, CH3O, trans), 3.54 (dd, J = 12.8, 4.3 Hz, 1H, δ-CH1, cis), 3.51 (dm, J = 11.7 Hz, 1H, δ-CH2, trans), 3.38 (dm, J = 12.8 Hz, 1H, δ-CH2, cis), 2.38-2.34 (m, 2 H, cis: β-CH2, 1H, trans: β-CH1), 2.12 (dm, J = 13.8 Hz, 1H, β-CH2, trans), 2.05 (s, 3H, CH3C=O, trans), 1.99 (s, 3H, CH3C=O, cis).

Ac-cis-MePro-OCH_3 ^{1}H NMR (D_2O, 600 MHz), only trans-conformer: 4.75 (m, 1H, α-CH), 3.64 (s, 3H, CH3O), 3.50 (dt, J = 6.2, 2.7 Hz , δ-CH), 2.61 (td, J = 12.9, 6.2 Hz, β-CH1), 2.17 (s, 3H, CH3C=O), 1.99 (dd, J = 12.9, 3.7 Hz, 1H, β-CH2), 1.71 (m, 1H, γ-CH), 0.84 and 0.71 (two m, 2H, CH2).

Ac-trans-MePro-OCH_3 ^{1}H NMR (D_2O, 600 MHz), only trans-conformer: 4.27 (dd, J = 9.7, 5.4 Hz, 1H, α-CH), 3.67 (s, 3H, CH3O), 3.47 (ddd, J = 6.6, 5.7, 2.4 Hz, 1H, δ-CH), 2.33 (ddd, J = 13.7, 9.5, 1.3 Hz, β-CH1), 2.21 (m, 1H, β-CH2), 2.16 (s, 3H, CH3C=O), 1.78 (m, 1H, γ-CH), 0.93 (dt, J = 8.7, 5.8 Hz, 1H, CH1), 0.58 (td, J = 5.5, 2.5 Hz, 1H, CH2).

Ac-3,4-Dhp-OCH_3 ^{1}H NMR (D_2O, 700 MHz): 6.04 (dm, J = 6 Hz, 1H, γ-CH, trans+cis), 5.82 (dm, J = 6 Hz, 1H, β-CH, cis), 5.78 (dm, J = 6 Hz, 1H, β-CH, trans), 5.33 (m, 1H, α-CH, cis), 5.09 (m, 1H, α-CH, trans), 4.38 (dm, J = 15.2 Hz, δ-CH1, trans), 4.34 (dm, J = 15.2 Hz, 1H, δ-CH2, trans), 3.74 (s, 3H, CH3O, cis), 3.69 (s, 3H, CH3O, trans), 2.07 (s, 3H, CH3C=O, trans), 1.94 (s, 3H, CH3C=O, cis).

Table S2: The pK_a values.

amino acid	**pK_a, amino group**[a]	**pK_a, carboxyl group**[b]		
	pK_a	**s-*trans***	**s-*cis***	**Weight Average**[c]
Pro	10.7	3.55	2.85	3.42
Aze	10.5	3.24	2.73	3.14
Pip	10.8	3.63	3.37	3.6
4-S-Flp	9.1	3.39	2.87	3.24
4-R-Flp	9.1	3.19	2.37	3.08
4,4-F_2-Pro	6.5	2.93	2.34	2.80
4-S-Hyp	10.0	3.62	3.19	3.49
4-R-Hyp	9.7	3.15	2.39	3.04
cis-MePro	9.6	3.47	2.84	3.37
trans-MePro	9.6	3.38	2.75	3.32
4-S-Mep	10.7	3.46	2.77	3.38
4-R-Mep	10.7	3.53	2.81	3.38
3,4-Dhp	9.8	3.03	2.37	2.93
Ala	9.9	3.56	3.11	3.56
Phe	9.3	3.42	2.98	3.42
Val	9.7	3.55	3.10	3.55

[a] values for the free amino acids determined in aqueous buffer at 298 K [10]; For comparison, previously reported amino-group pK_a values for Pro, 4-R-Flp, 4,4-F_2-Pro, and 4-R-Hyp, are 10.8, 9.2, 7.2, and 9.7, respectively [5 2]; [b] pK_a values of the carboxyl-group of Ac-Pro* are taken from [9]. [c] The weight average pK_a was calculated from the *cis* and *trans* pK_as taken the *cis*-*trans* equilibrium into account (Table S1).

Table S3: Rate of reactions for Pro and Pro-derivatives

Pro*	k_{hydrol}	k_{aminol}	fMP*-Pmn		fMP*G		fMP*P*G	
			no	EF-P	no	EF-P	no	EF-P
	$\times 10^{-5}$ s^{-1}	$\times 10^{-5}$ s^{-1}	k_{pep}, s^{-1}	k_{pep}, s^{-1}	k_{obs}, s^{-1}	k_{obs}, s^{-1}	k_{obs}, s^{-1}	k_{obs}, s^{-1}
Pro	6.3 ± 0.5	3.7 ± 0.5	0.14 ± 0.1	8.2 ± 0.8	4.2 ± 0.3	33 ± 2	0.018 ± 0.02	0.6 ± 0.02
Aze			0.55 ± 0.26 (40%)	45 ± 13 (34%)				
			0.12 ± 0.04 (60%)	2.4 ± 0.3 (66%)				
Pip			0.5 ± 0.06 (68%)	27 ± 3 (81%)				
			0.03 ± 0.01(32%)	0.7 ±0. 3 (19%)				
4-S-Flp	3 ± 0.3	2 ± 0.4	0.003 ± 0.0002	0.23 ± 0.02	0.15 ± 0.02	5 ± 0.5	no product	0.013 ± 0.001
4-R-Flp	23 ± 1	9 ± 3	21 ± 2	121 ± 33	73 ± 10	63 ± 9	0.22 ± 0.02	0.53 ± 0.06
4,4-F_2-Pro	31 ± 1	28 ± 5	2.3 ± 0.2	67 ± 5	42 ± 4 (73%)	65 ± 14 (83%)	0.077 ± 0.005	0.7 ± 0.1
					0.07 ± 0.02 (27%)	0.3 ± 0.3 (17%)		
4-S-Hyp	14 ± 1	4 ± 1	0.007 ± 0.0003	0.64 ± 0.06	0.3 ± 0.03	11 ± 1	0.004 ± 0.001	0.077 ± 0.008
4-R-Hyp	6.3 ± 0.1	7 ± 2	0.2 ± 0.02	9.4 ± 0.5	4 ± 0.3	49 ± 7	0.039 ± 0.004	0.3 ± 0.04
***Cis*-MePro**	1.7 ± 0.2	1.8 ± 0.3	6 ± 1 × 10^{-5}	0.001 ± 0.0004	0.001 ± 0.0001	0.037 ± 0.003	no product	0.013 ± 0.002
***Trans*-MePro**	8.4 ± 0.3	6.6 ± 0.6	0.32 ± 0.03	14 ± 2	28 ± 4 (68%)	69 ± 11 (86%)	0.065 ± 0.008	0.64 ± 0.09
					1.7 ± 0.5 (32%)	0.6 ± 0.5 (14%)		
4-S-Mep	3.7 ±0.9		0.09 ± 0.002 (21%)	6 ± 1(38%)	9 ± 4 (32%)	26 ± 9 (72%)	0.009 ± 0.004	0.085 ± 0.008
			0.003 ± 0.0002 (79%)	0.18 ± 0.03 (62%)	0.16 ± 0.03 (68%)	1 ± 1 (28%)		
4-R-Mep	3.8 ±0.04	10 ± 1	0.17 ± 0.03	12 ± 1	19 ± 3 (69%)	73 ± 9 (75%)	0.072 ± 0.006	0.51 ± 0.05
					0.6 ± 0.2 (31%)	1.9 ± 0.6 (25%)		
3,4-Dhp	5.6 ± 0.2	4.4± 1	0.01 ± 0.001	0.62 ± 0.07	2.8 ± 0.4 (67%)	21 ± 4 (82%)	0.005 ± 0.001	0.12 ± 0.01
					0.23 ± 0.07 (33%)	0.2 ± 0.1 (18%)		
fMet	11 ± 0.6	11± 1						

Table S4: Activation parameters of the Pmn reaction with fMet-R/S-Flp-tRNA^{Pro} for 37°C

Pro*, EF-P	**$\Delta G^{\neq}$**, kcal/mol	**$\Delta H^{\neq}$**, kcal/mol	**$T\Delta S^{\neq}$**, kcal/mol
4-R-Flp, no EF-P	16 ± 1	22 ± 2	6.4 ± 0.6
4-R-Flp, EF-P	15 ± 1	24 ± 1	8.7 ± 0.7
4-S-Flp, no EF-P	22 ± 1	19 ± 2	- 2.3 ± 0.2
4-S-Flp, EF-P	19 ± 1	24 ± 1	4.6 ± 0.3

Calculated for 37 °C, $\Delta H^{\neq} = E_a - RT$, $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$, $T\Delta S^{\neq}_{(37°C)} = T\Delta S^{\neq}_{(25\,°C)} * 310.15K/298.15K$

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