

Supporting Information for

A cascade of structural rearrangements positions peptide release factor II for polypeptide hydrolysis on the ribosome

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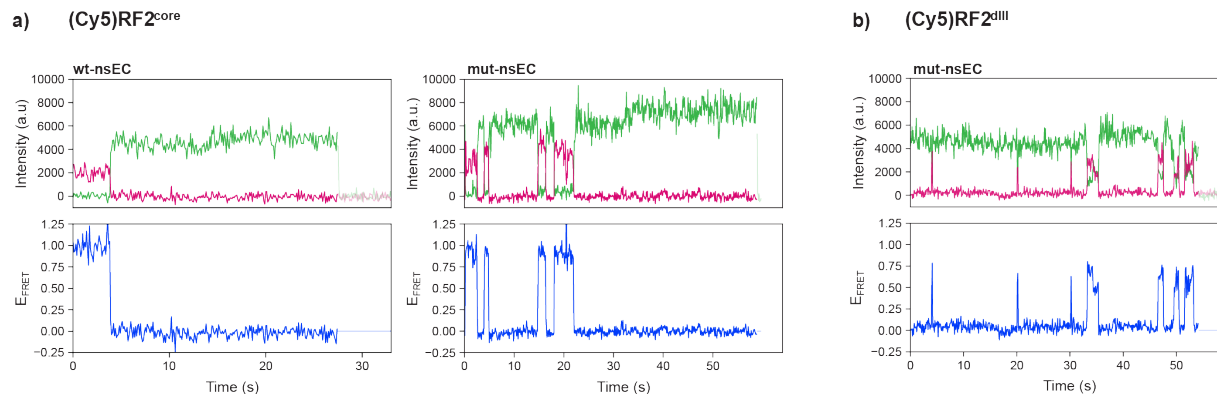


Figure S1. smFRET signals for RF2 stable association and dynamics. Representative smFRET trajectories showing the Cy3 (green) and Cy5 (red) fluorescence intensities and the corresponding E_{FRET} (blue) for **(a)** wt-nsEC (left) and mut-nsEC (right) with the (Cy5)RF2^{core} construct and **(b)** mut-nsEC with the (Cy5)RF2^{dIII} construct.

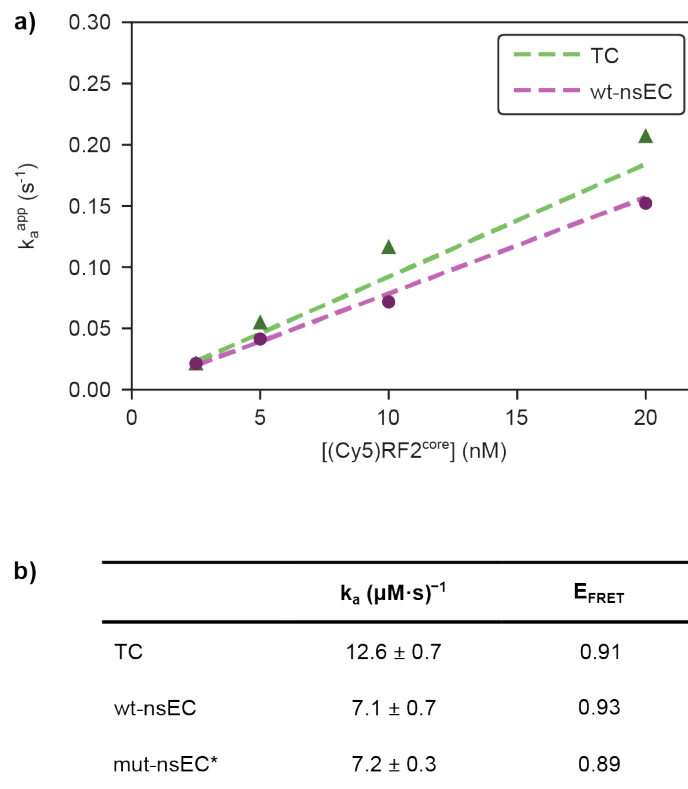


Figure S2. Kinetics of RF2 stable association. **(a)** First-order k_a^{app} for $(\text{Cy5})\text{RF2}^{\text{core}}$ association with TCs and ArfA-bound wt-nsECs, and **(b)** second-order k_a for RF2^{core} association with TCs and ArfA-bound wt-nsECs and mut-nsECs (*estimated from steady-state experiments).

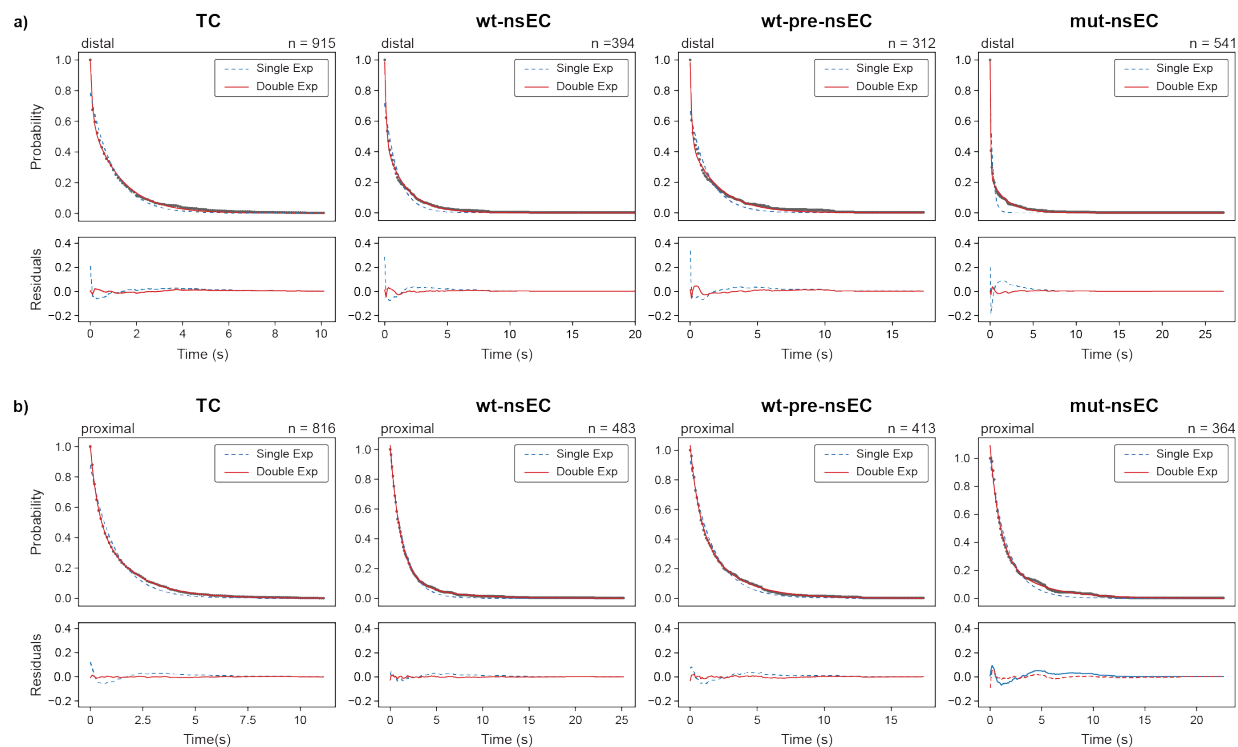


Figure S3. Dwell analysis of the extended and collapsed conformations of ribosome-bound (Cy5)RF2^{dIII}. Survival plots of the dwell time distributions (black) along with fits of single-exponential (blue) and double-exponential (red) decays (above) and the corresponding residuals (below) for **(a)** the distal state, and **(b)** the proximal state for different ribosomal complexes.

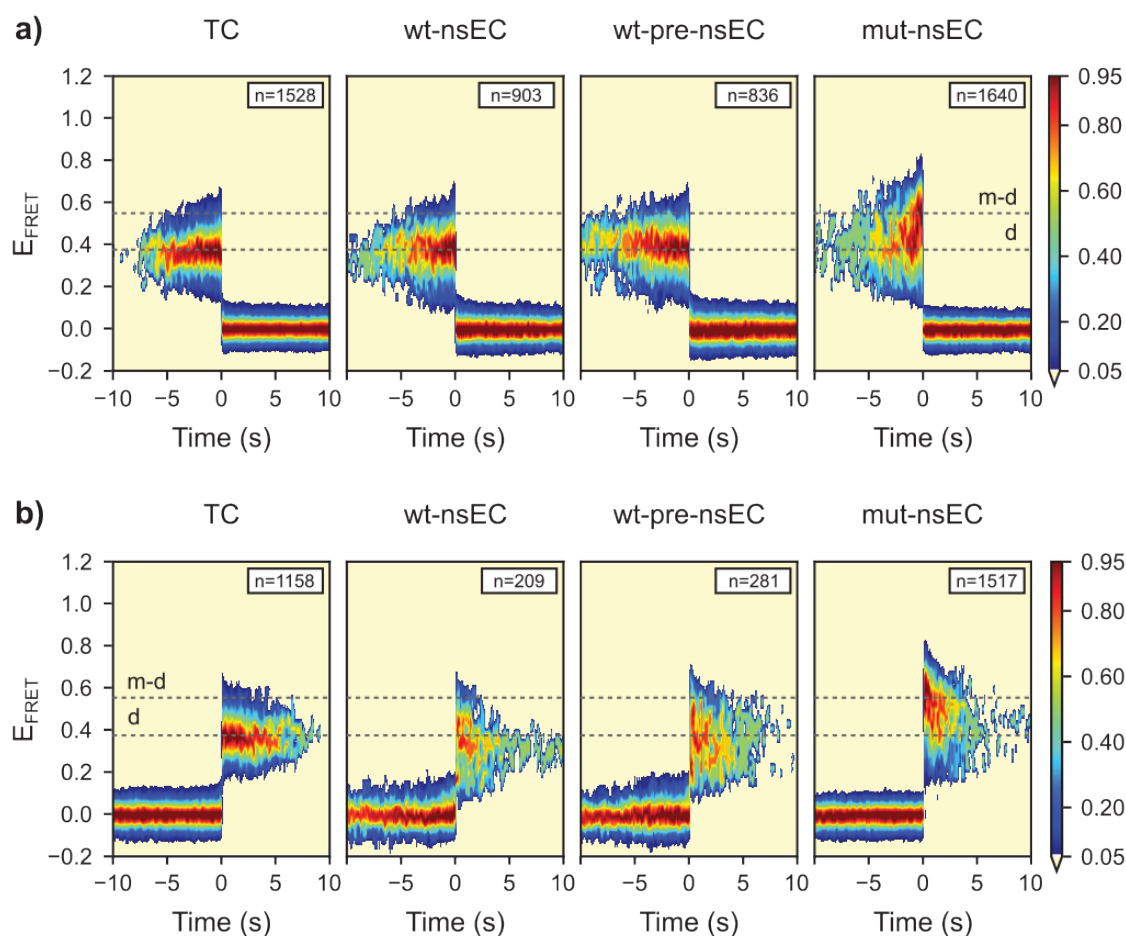


Figure S4. 2D E_{FRET} histograms of RF2 dissociation and stable association. 2D histograms post-synchronized to **(a)** the transition from the distal E_{FRET} state to the zero E_{FRET} state, following the dissociation of ribosome-bound (Cy5)RF2^{dIII}, and **(b)** the transition from the zero E_{FRET} state to the distal E_{FRET} state, following the stable association of (Cy5)RF2^{dIII} to the ribosome, for different ribosomal complexes. Grey dashed lines show the E_{FRET} states corresponding to the distal (d), and transient mid-distal (m-d) conformations of (Cy5)RF2^{dIII}.

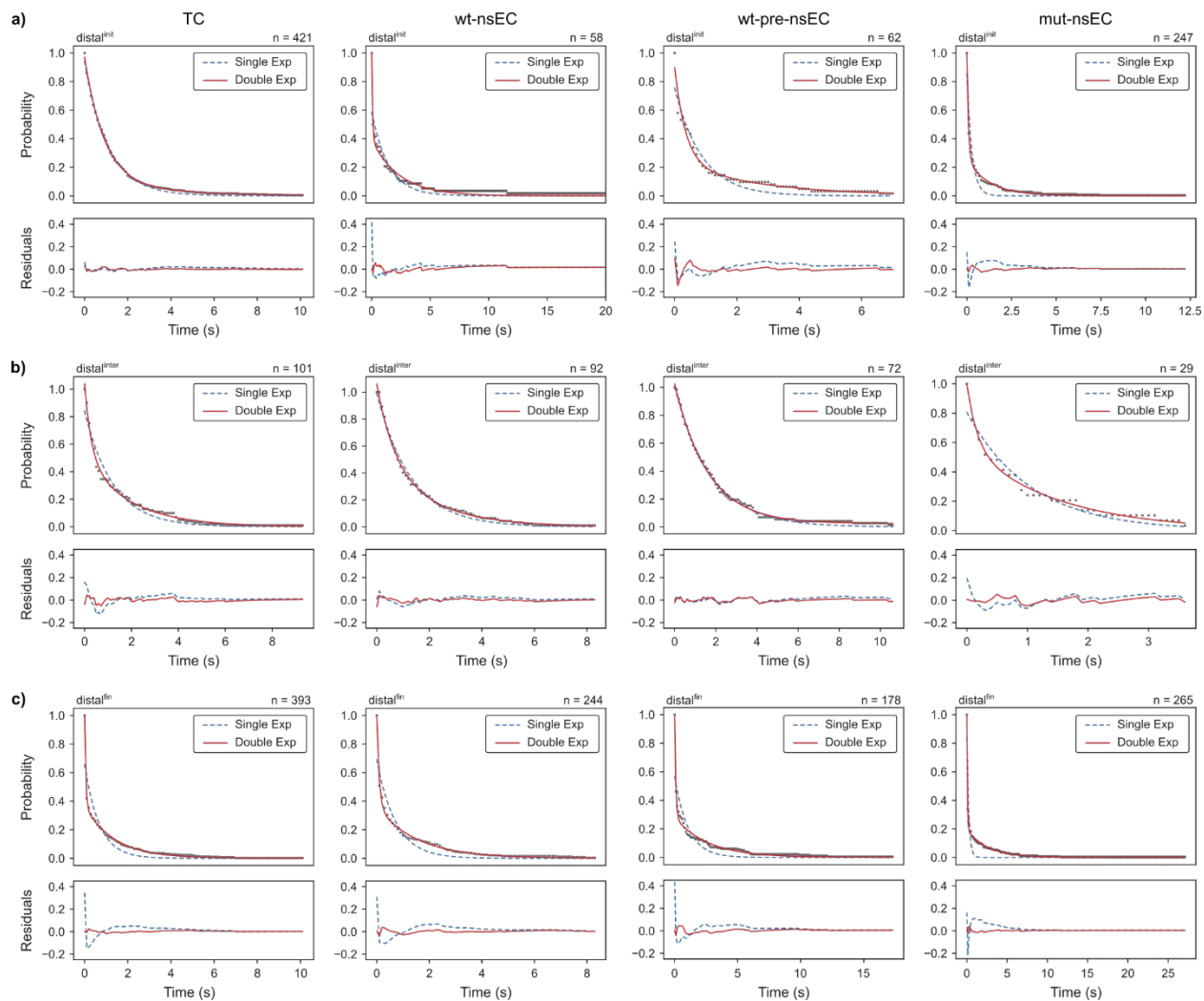


Figure S5. Parsed dwell analysis of the extended conformation of ribosome-bound (Cy5)RF2^{dlII}. Survival plots of the dwell time distributions (black) along with fits of single-exponential (blue) and double-exponential (red) decays (above) and the corresponding residuals (below) for **(a)** $distal^{init}$ **(b)** $distal^{inter}$, and **(c)** $distal^{fin}$, for different ribosomal complexes.

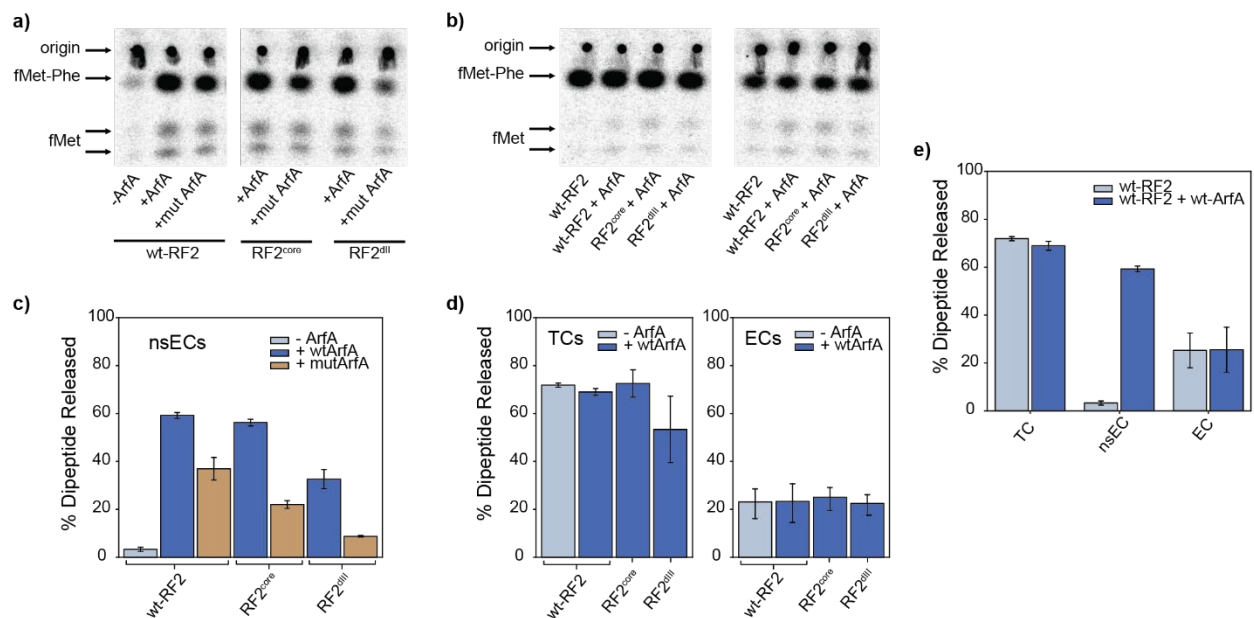


Figure S6. fMet-Phe dipeptide release assay. (a) representative eTLC of fMet-Phe dipeptide released from nsEC complexes incubated with wt-RF2, (Cy5)RF2^{core}, or (Cy5)RF2^{dll} and either no ArfA (for wt-nsEC only), wt-ArfA, or mut-ArfA after one minute at 37°C; (b) representative eTLC of fMet-Phe dipeptide released from TC or EC complexes incubated with wt-RF2, (Cy5)RF2^{core}, or (Cy5)RF2^{dll}, with (for wt-RF2 only) and without wt-ArfA, for one minute at 37°C; (c) % dipeptide released from nsECs, quantified from eTLC analyses performed as in (a); (d) % dipeptide released from ECs and TCs, quantified from eTLC analyses performed as in (b); (e) comparison of % dipeptide released from TCs, nsECs, and ECs with wt-RF2, with or without wt-ArfA, from eTLC analyses performed as in a) and b).

Table S1. E_{FRET} s observed for all complexes. E_{FRET} s obtained from our HMM-based analysis for complexes with (Cy5)RF2^{core} and complexes with (Cy5)RF2^{dIII}. Errors represent the standard error of the mean, followed by the standard deviation about the mean (SD), as derived from our HMM analysis.

	(Cy5)RF2 ^{core}	(Cy5)RF2 ^{dIII} proximal	(Cy5)RF2 ^{dIII} distal
TC	0.91 ± 0.009 SD = 0.22	0.76 ± 0.009 SD = 0.11	0.37 ± 0.006 SD = 0.10
wt-nsEC	0.93 ± 0.015 SD = 0.17	0.70 ± 0.009 SD = 0.12	0.36 ± 0.007 SD = 0.11
wt-pre-nsEC	0.91 ± 0.015 SD = 0.18	0.69 ± 0.009 SD = 0.13	0.37 ± 0.007 SD = 0.11
mut-nsEC	0.89 ± 0.009 SD = 0.15	0.75 ± 0.009 SD = 0.12	0.43 ± 0.009 SD = 0.16
Api137-bound TC	-	-	0.39 ± 0.004 SD = 0.10

Table S2. Rates obtained from parsed dwell analysis of the extended conformation of ribosome-bound (Cy5)RF2^{dIII}. Rates, amplitudes, and R² values obtained from our parsed dwell analysis. Note that for wt-nsEC distal^{init}, the R² for the double exponential fit is 0.94, which is lower than our threshold value of 0.95. However, the single exponential fit was significantly worse, with an R² value of 0.84; thus, a double exponential fit was assigned in this case.

		k_1 (s ⁻¹)	k_2 (s ⁻¹)	A ₁	A ₂	R ²
TC	distal ^{init}	0.09	-	0.94	-	0.99
	distal ^{inter}	0.08	-	0.84	-	0.96
	distal ^{fin}	0.07	1.97	0.36	0.63	0.99
wt-nsEC	distal ^{init}	0.04	1.36	0.39	0.60	0.94*
	distal ^{inter}	0.07	-	0.99	-	0.99
	distal ^{fin}	0.06	1.10	0.36	0.63	0.99
wt-pre-nsEC	distal ^{init}	0.04	0.28	0.24	0.66	0.97
	distal ^{inter}	0.06	-	1.00	-	0.99
	distal ^{fin}	0.04	1.13	0.28	0.71	0.98
mut-nsEC	distal ^{init}	0.08	1.18	0.25	0.74	0.99
	distal ^{inter}	0.07	0.59	0.57	0.42	0.99
	distal ^{fin}	0.04	1.55	0.20	0.80	0.99

* highest R² value obtained for this complex