

Supplementary materials for

*N*⁶-methyladenosines in mRNAs reduce the accuracy of codon reading by transfer RNAs and peptide release factors

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Supplementary Figures

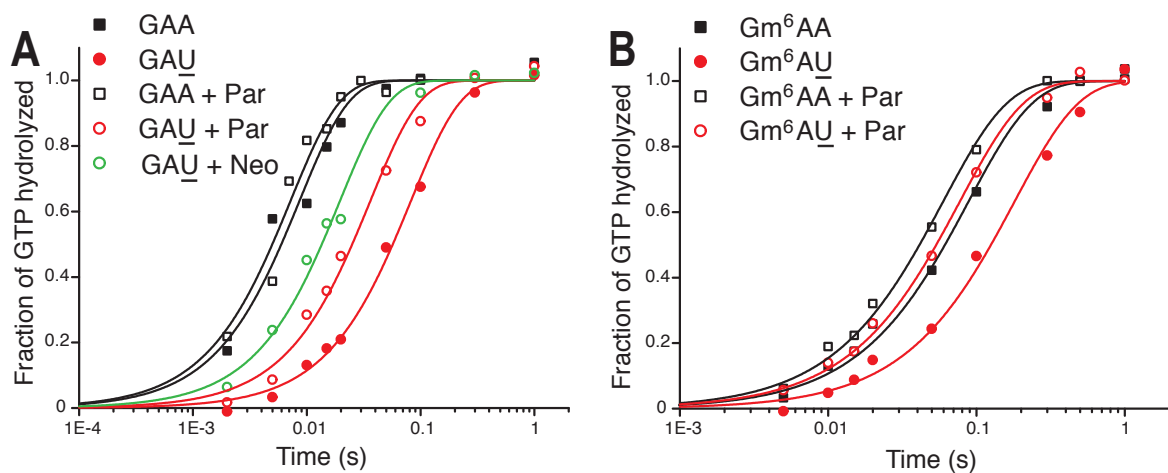


Figure S1. *m*⁶A modifications and the impact of aminoglycoside addition on the efficiency of codon reading by *T*₃ at high [Mg²⁺] concentration. The experiments were carried out as described in Figure 2 of main text with ribosomes (0.6 μM) in excess over ternary complex (*T*₃). Fractions of GTPs hydrolyzed on Glu-tRNA^{Glu}-containing *T*₃ (*y*-axis) at different reaction times (*x*-axis, log₁₀ display) for *T*₃ reading unmodified codons at 25 mM (A) and modified codons at 16 mM (B) free [Mg²⁺]. Cognate GAA (A) or Gm⁶AA (B) closed black squares in the absence and open black squares in the presence of Par. Near cognate GAU (A) or Gm⁶AU (B) filled red circles in the absence and open red circles in the presence of Par. Near cognate GAU open green circles in the presence of Neo (A).

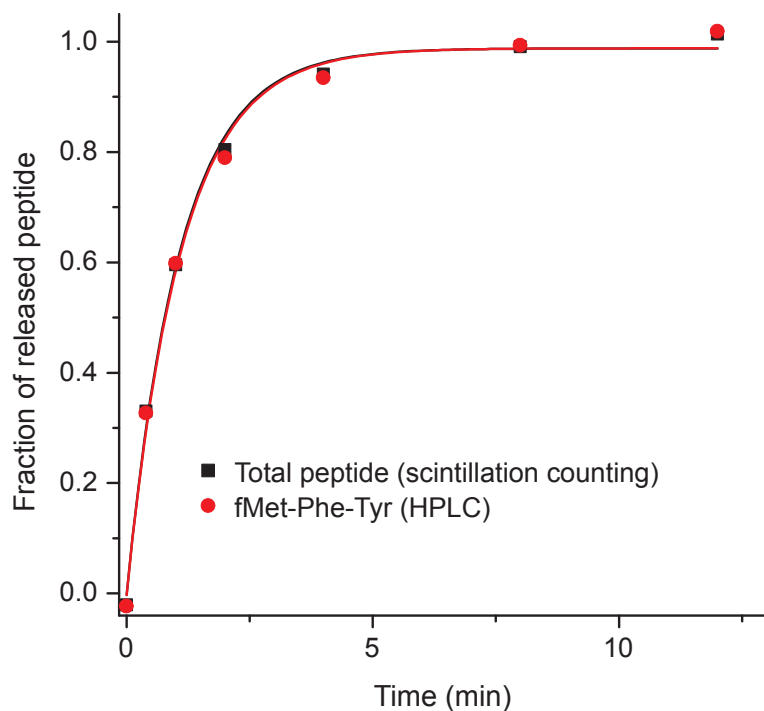


Figure S2. A comparison of peptide release measured by scintillation counting (total peptide) and HPLC (only fMFY). 0.3 μM ribosomal termination complexes containing ^3H -labelled fMet-Phe-Tyr-tRNA^{Tyr} in the P site and UAG codon in the A site were reacted to 3.3 μM RF2 in polymix buffer with 2.5 mM free Mg^{2+} at 37 $^{\circ}\text{C}$. The k_{rel} measured by HPLC and by scintillation counting was the same (0.9 ± 0.06 /min and 0.9 ± 0.04 /min respectively).

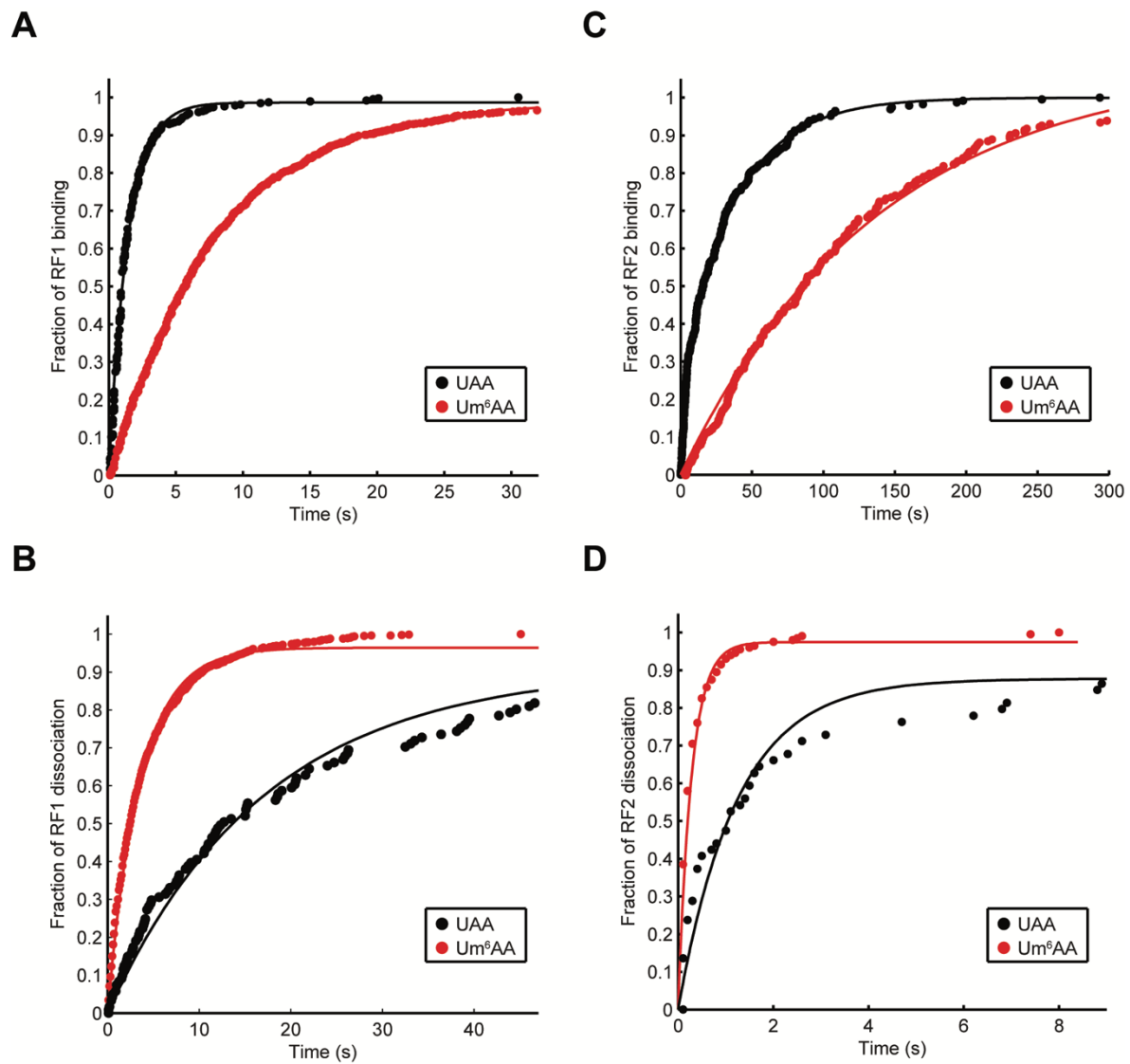


Figure S3. Measurements of RF1 and RF2 association and dissociation kinetics on UAA and Um⁶AA codons from single-molecule assay. (A) Time-course of RF1 arrival on UAA and Um⁶AA codons fit to single exponential functions. (B) Time-course of RF1 dissociation from UAA and Um⁶AA codons fit to single exponential functions. (C) Time-course of RF2 arrival on UAA and Um⁶AA codons fit to single (Um⁶AA) and double (UAA) exponential functions. (D) Time-course of RF2 dissociation from UAA and Um⁶AA codons fit to single exponential functions. All time-course plots are cumulative distribution functions generated from measured RF arrival and RF occupancy times (see Figure 8C-D).

Supplementary Tables

Table S1. Accuracy parameters for tRNA^{Glu} selection on modified and unmodified codons with and without the aminoglycoside paromomycin. Measurements were conducted in polymix buffer with 2.3 mM free Mg²⁺. Related to Figure 3.

Codon pairs	Paromomycin	k_a ($\mu\text{M}^{-1}\text{s}^{-1}$)	d_e value	Initial selection	Proofreading	Total accuracy
GAA/GAU	No	239 ± 10	80 ± 4	60 ± 10	290 ± 40	17500 ± 2600
GAA/GAU	10 μM	260 ± 15	11 ± 1			
Gm ⁶ AA/Gm ⁶ AU	No	20 ± 9	20 ± 13	15 ± 1.6	92 ± 8	1360 ± 170
Gm ⁶ AA/Gm ⁶ AU	10 μM	22 ± 9	2.8 ± 1.2			

Table S2. Kinetic parameters for tRNA^{Glu} reading its cognate codons. Measurements were conducted in polymix buffer with 2.3 mM free Mg²⁺. Related to Figure 4A,B.

Codon	k_{GTP} (s^{-1})	Rib (μM)	k_{cat}/K_m (GTP) ($\mu\text{M}^{-1}\text{s}^{-1}$)	k_{dip} (s^{-1})	k_{pep} (s^{-1})	Yield of dipeptide (μM)
GAA	120 ± 15	2	59 ± 7	50 ± 5	86 ± 18	0.65
Gm ⁶ AA	12 ± 0.6	2	5.9 ± 0.3	10 ± 0.3	58 ± 30	0.63

Table S3. Kinetic parameters for dipeptide formation from tRNA^{Glu} misreading GAU and Gm⁶AU. Related to Figure 4C, D. T₃ - ternary complex EF-Tu·Glu-tRNA^{Glu}·GTP.

Codon	T ₃ (μM)	k_{dip} (ms^{-1})	k_{cat}/K_m ($\text{mM}^{-1}\text{s}^{-1}$)	Average k_{cat}/K_m ($\text{mM}^{-1}\text{s}^{-1}$)
GAU	0.7	2.2 ± 0.3	3.2 ± 0.4	3.4 ± 0.3
GAU	1.4	5.0 ± 0.7	3.6 ± 0.5	
Gm ⁶ AU	0.7	2.7 ± 0.3	3.9 ± 0.4	4.1 ± 0.3
Gm ⁶ AU	1.4	6.1 ± 0.6	4.4 ± 0.4	

Table S4. Kinetic parameters for measurement of apparent dissociation constant for tRNA^{Glu} reading its cognate codons.

Codon	A_{slow}/A_{fast}	k_{slow} (s ⁻¹)	k_{dis} (s ⁻¹)
GAA	1.1	1.1 ± 0.2	2.4 ± 0.3
Gm ⁶ AA	0.84	0.76 ± 0.17	1.4 ± 0.3