Supplementary Data for

Thio-modification of tRNA at the wobble position as regulator of the kinetics of decoding and translocation on the ribosome

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Table S1. Summary of the elemental rate constants and goodness of the global fits.

Mean values with S.E.M. were calculated by global fitting of the datasets of Figures S1 and S2 with KinTek Global Kinetic Explorer using the model described in Figure 2. The calculated χ^2 threshold was 1.00296 for $mcm^5s^2U_{34}$ and 1.00285 for mcm^5U_{34} $tRNA^{Lys}$, which corresponds to a 0.296% and 0.285% error of fitted parameters, respectively. To obtain a more conservative estimate for the rate constants, we increased the percent error by a factor of 4 and calculated the lower and upper boundaries for the rate constants from the confidence plots applying a χ^2 threshold of 1.01. n.d., not defined.

Rate constant	**Mean with S.E.M.**	**Lower Boundary**	**Upper Boundary**
$mcm^5s^2U_{34}$ $tRNA^{Lys}$			
k_1, $\mu M^{-1}s^{-1}$	130 ± 7	117	141
k_{-1}, s^{-1}	132 ± 8	106	166
k_2, s^{-1}	60 ± 3	47	74
k_{-2}, s^{-1}	4 ± 0.5	3	5
k_3, s^{-1}	>500	n.d.	n.d.
k_4, s^{-1}	13 ± 1	10	16
k_{5a}, s^{-1}	1.3 ± 0.1	1.2	1.5
k_{5b}, s^{-1}	0.2 ± 0.04	0.1	0.8
k_{6a}, s^{-1}	0.8 ± 0.1	0.6	1
k_{6b}, s^{-1}	1.4 ± 0.1	1.2	1.5
k_7, s^{-1}	1 ± 0.1	0.8	1.3
mcm^5U_{34} $tRNA^{Lys}$			
k_1, $\mu M^{-1}s^{-1}$	162 ± 8	144	190
k_{-1}, s^{-1}	152 ± 8	121	200
k_2, s^{-1}	53 ± 2	43	67
k_{-2}, s^{-1}	22 ± 3	18	28
k_3, s^{-1}	>500	n.d.	n.d.
k_4, s^{-1}	2 ± 0.2	1.2	2
k_{5a}, s^{-1}	1.1 ± 0.3	0.6	3.8
k_{5b}, s^{-1}	0.1 ± 0.01	0.05	0.38
$k_{6a,}$ s^{-1}	n.d.	n.d.	n.d.
k_{6b}, s^{-1}	1.2 ± 0.3	0.6	3.8
k_7, s^{-1}	3 ± 0.3	2.0	3.5

Table S2. Rate constants of EF-G-induced subunit rotation and tRNA translocation.

$tRNA^{Lys}$	$k_{app1\ CCW}$, s^{-1}	$k_{app2\ CW}$, s^{-1}	$k_{app\ Pmn}$, s^{-1}
mcm^5s^2	143 ± 20	3.4 ± 0.1	2 ± 0.2
mcm^5	133 ± 15	1.3 ± 0.1	1 ± 0.1

SUPPLEMENTARY FIGURES

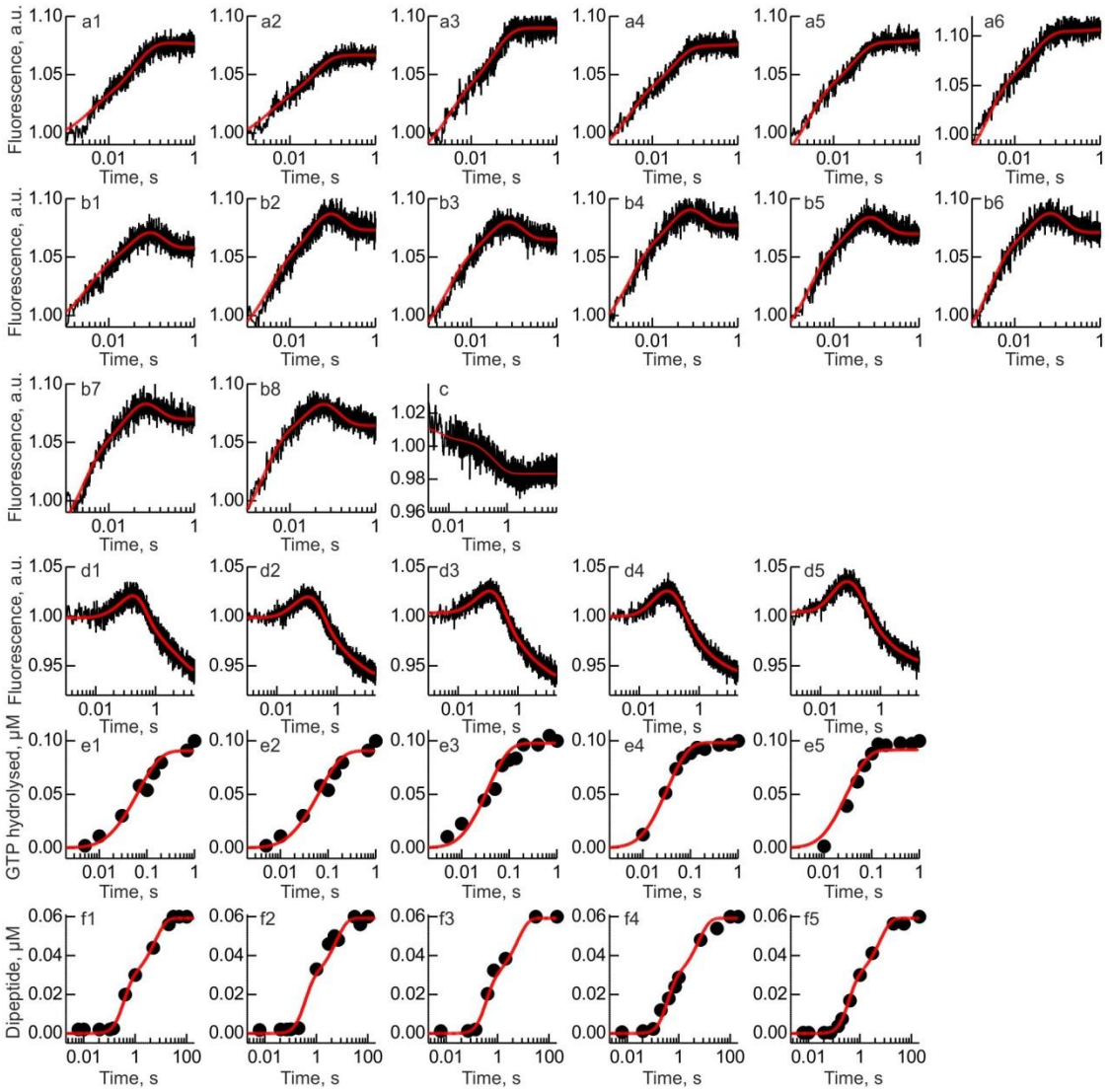

Figure S1. Global fitting of time courses for mcm^{5}s^{2}U$_{34}$ tRNA^{Lys}.

The same concentration of the ternary complex EF-Tu–GTP–Lys-tRNA^{Lys} (0.1 μM) was used in all experiments. (a) Fluorescence changes of [^{14}C]Lys-tRNA^{Lys}(Prf16/17) reporting initial binding. Time courses a1-a6 were recorded at 0.8, 1.0, 1.2, 1.5, 1.8 and 2.0 μM of vacant ribosomes. (b) Conformational changes of [^{14}C]Lys-tRNA^{Lys}(Prf16/17) upon decoding of the cognate AAA codon. Time courses b1-b8 were obtained at 0.6, 0.8, 1.1, 1.2, 1.4, 1.5, 1.8 and 2.0 μM of 70S–mRNA–f[^{3}H]Met-tRNA^{fMet} initiation complex. (c) Dissociation from the codon recognition complex. The ternary complex EF-Tu(H84A)–GTP–[^{14}C]Lys-tRNA^{Lys}(Prf16/17) was chased with 10-fold excess ternary complex containing non-fluorescent Lys-tRNA^{Lys}. (d) Conformational changes of EF-Tu monitored as changes of mant-GTP/GDP fluorescence. Time courses d1-d5 were obtained at 0.5, 0.75, 1.0, 1.5 and 2.0 μM of initiation complex. (e) GTP hydrolysis. Time courses e1-e5 were obtained at 0.5, 1.0, 1.2, 1.4 and 1.6 μM of initiation complex. (f) Peptide bond formation. Time courses f1-f5 were obtained at 0.8, 1.0, 1.4, 1.8 and 2.0 μM μM of initiation complex.

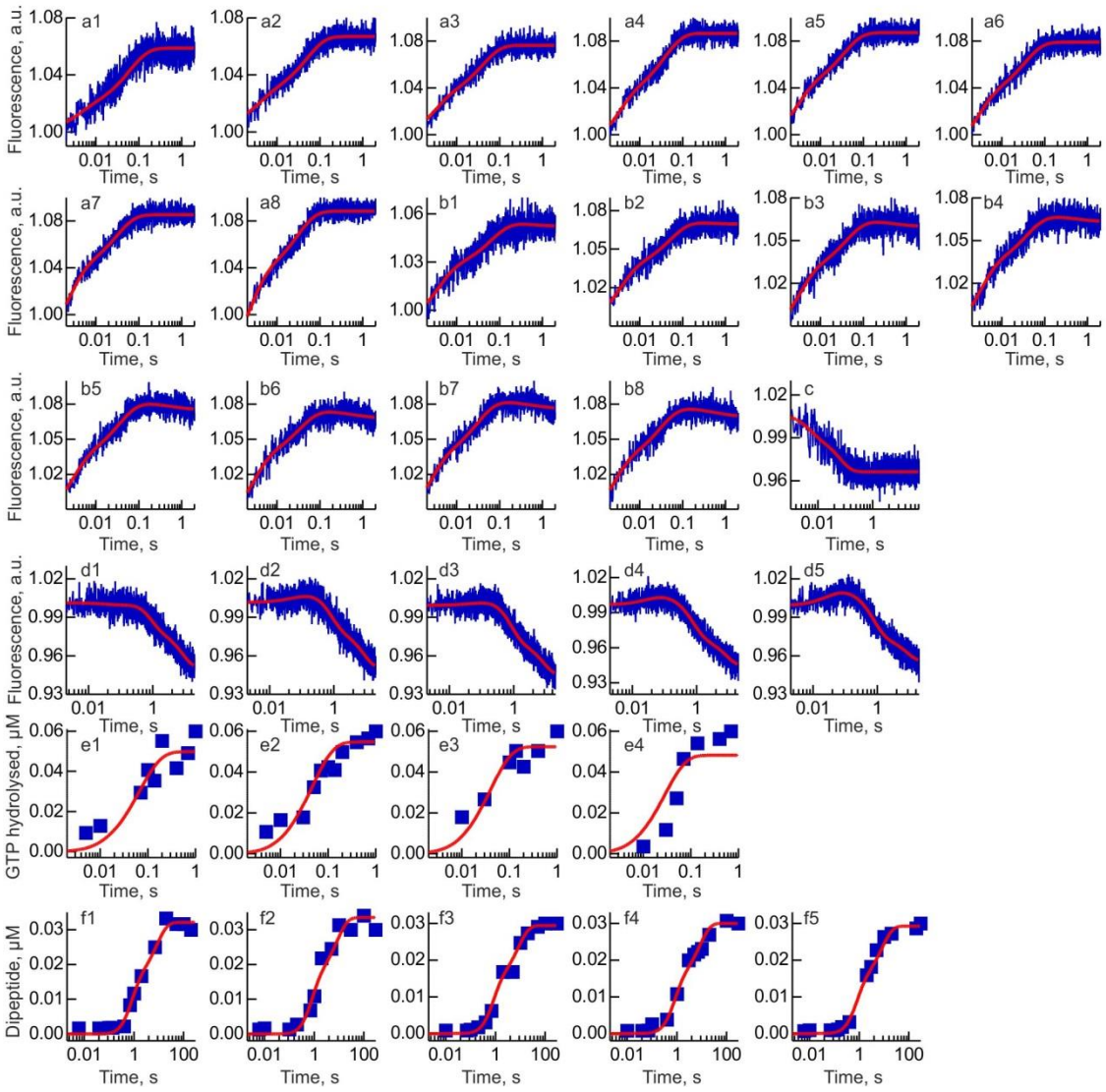

Figure S2. Global fitting of time courses for mcm^{5}U$_{34}$ tRNA^{Lys}.

The same concentration of the ternary complex EF-Tu–GTP–Lys-tRNA^{Lys} (0.1 μM) was used in all experiments. (a) Fluorescence changes of [^{14}C]Lys-tRNA^{Lys}(Prfi6/17) reporting initial binding. Time courses a1-a8 were recorded 0.6, 0.8, 1.1, 1.2, 1.4, 1.5, 1.9 and 2.0 μM of vacant ribosomes. (b) Conformational changes of [^{14}C]Lys-tRNA^{Lys}(Prfi6/17) upon decoding of the cognate AAA codon. Time courses b1-b8 were obtained at 0.6, 0.8, 1.1, 1.2, 1.4, 1.5, 1.9 and 2.0 μM of 70S–mRNA–f[^{3}H]Met-tRNA^{fMet} initiation complex. (c) Dissociation from the codon recognition complex. The ternary complexes EF-Tu(H84A)–GTP–[^{14}C]Lys-tRNA^{Lys}(Prfi6/17) was chased with 10-fold excess ternary complex with non-fluorescent Lys-tRNA^{Lys}. (d) Conformational changes of EF-Tu monitored as changes of mant-GTP/GDP fluorescence. Time courses d1-d5 were obtained at 0.5, 0.75, 1.0, 1.5 and 2.0 μM of initiation complex, respectively. (e) GTP hydrolysis. Time courses e1-e4 were obtained at 0.5, 0.8, 1.0 and 2.0 μM of initiation complex. (f) Peptide bond formation. Time courses f1-f5 were obtained at 0.8, 1.0, 1.4, 1.8 and 2.0 μM of initiation complex.

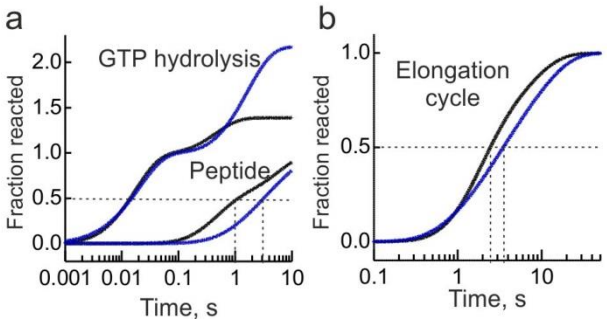

Figure S3. Simulations of the decoding reactions and the ribosome residence times on a Lys codon with a fully modified (black) or s^2-hypomodified (blue) Lys-$tRNA^{Lys}$.

Simulations were carried out in KinTek Explorer using the elemental rate constants given in Tables S1 and S2. Concentration of ribosomes at Lys codons was set to 1 μM (5% of total ribosome concentration). Ternary complexes were assumed to be in excess (3 μM; modeling with 10 μM gives the same result). (a) Effect on GTP hydrolysis (upper curves) and peptide formation (lower curves). (b) The overall effect on the duration of the elongation cycle, including decoding, peptide bond formation and translocation. Dashed lines indicate the times required to complete the reaction on half of the ribosomes, or half-life of ribosome residence on a Lys codon.