SUPPLEMENTARY MATERIAL FOR

Natural amino acids do not require their native tRNAs for efficient selection by the ribosome

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Supplementary Methods

Buffer system

Translation reactions were run in Tris-polymix buffer containing 50 mM Tris-OAc ($pH_{25°C}$=7.5), 100 mM KCl, 5 mM NH_4OAc, 0.5 mM $Ca(OAc)_2$, 3.5 mM $Mg(OAc)_2$, 6 mM 2-mercaptoethanol, 1% glucose, 5 mM putrescine, and 1mM spermidine[1].

Preparation of highly-purified in vitro translation components

Tightly coupled *E. coli.* 70S ribosomes were purified[2] and stored in 10 mM Tris-OAc ($pH_{4°C}$=7.5), 60 mM NH_4Cl, 7.5 mM $Mg(OAc)_2$, 0.5 mM EDTA, 6 mM 2-mercaptoethanol and ~35% sucrose at -80 ºC. Initiation factors 1, 2 and 3, elongation factors Tu and Ts, formylmethionyl-tRNA formyltransferase and methionyl-tRNA synthetase were purified as previously described.[1] Phenylalanyl-tRNA synthetase was purified from an overexpression strain kindly provided by Prof. David Tirrell[3]. Alanyl-tRNA synthetase was PCR-cloned from *E. coli* C600 genomic DNA by Mr. Noam Prywes and inserted into the pProEX-HTb expression plasmid, which introduces a N-terminal, six-histidine affinity tag that is subsequently cleaved from the synthetase with TeV protease prior to use.[1]

Preparation of mRNAs

mRNAs used for dipeptide yield and competition experiments contained a strong (UAAGGAGGU) Shine-Dalgarno sequence, an epsilon enhancer sequence, and three codons encoding fMet (**7**), either Ala, Lys, or Phe, and Glu. These mRNAs were made by run-off transcription of PCR-extended oligonucleotides using a purified T7 RNA

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polymerase. Transcribed mRNAs were subsequently purified by denaturing polyacrylamide gel electrophoresis. Control experiments were performed with a longer mRNA similar to that used for the smFRET experiments to test for mRNA length dependence. mRNA length did not affect selectivity (Supplementary Fig 10). The mRNA used in the smFRET experiments is a T4 gene product 32-derived mRNA which was made by run-off transcription of a linearized plasmid DNA template. The 5'-end of this mRNA contains an 18 nucleotide hybridization sequence that is followed by a 6 nucleotide spacer sequence, a strong Shine-Dalgarno (UAAGGA) sequence, nucleotides encoding fMet, Phe, and Lys as the first three amino acids, and nucleotides encoding an additional 220 amino acids from the T4 gene product 32 sequence[1]. Biotinylation at the 5'-end of this run-off transcribed mRNA was achieved by hybridization of a 3'-biotinylated, 18 nucleotide DNA oligonucleotide (IDT, Inc) complementary to the 18 nucleotide hybridization sequence at the 5'-end of the mRNA.

Purification, fluorescent-labeling, enzymatic aminoacylation, and formylation of tRNAs

$tRNA^{fMet}$ and $tRNA^{Phe}$ were purchased from Sigma, Inc. and $tRNA^{Phe}$ was further purified by hydrophobic interaction chromatography. $tRNA^{Ala}$ was purified from an *E. coli* total tRNA pool (Roche, Inc.) as described above. For smFRET experiments, $tRNA^{fMet}$ was labeled with a maleimide derivative of Cy3 (GE Life Sciences, Inc.) at the 4-thiouridine at nucleotide position 8 and $tRNA^{Phe}$ was labeled with an *N*-hydroxysuccinimidyl ester derivative of Cy5 (GE Life Sciences, Inc) at the 3-(3-amino-3-carboxy-propyl) uridine at nucleotide position 47 as previously described[1].

fMet-$tRNA^{fMet}$, fMet-(Cy3)$tRNA^{fMet}$, Phe-$tRNA^{Phe}$, Phe-(Cy5)$tRNA^{Phe}$ were formylated and/or aminoacylated using purified formylmethionyl-tRNA formyltransferase, methionyl-tRNA synthetase, and phenylalanyl-tRNA synthetase as previously reported.[1] All other aminoacyl-tRNAs were aminoacylated with the dFx Flexizyme ribozyme and dinitrobenzyl ester activated amino acids[4].

Phenylalanine aminoacylation efficiencies using $tRNA^{Phe}$ were measured using hydrophobic interaction chromatographic separation of Phe-$tRNA^{Phe}$ from deacylated $tRNA^{Phe}$. Methionine aminoacylation and formylation efficiencies using $tRNA^{fMet}$ were determined using acidic denaturing polyacrylamide gel electrophoretic separation of fMet-$tRNA^{fMet}$ from deacylated $tRNA^{fMet}$. All other aminoacylation efficiencies were estimated using thin layer chromatography (please see below).

70S initiation complex formation

70S initiation complexes were *in vitro* initiated in Tris-polymix buffer using slight modifications of previously reported protocols.[1,5] The initiation reaction yields 70S initiation complexes containing final concentrations of 0.86 μM tightly-coupled 70S ribosomes, 1 μM IF1, 1.3 μM IF2, 1.1 μM IF3, 1 mM GTP, 1.8 μM mRNA and 1 μM fMet-$tRNA^{fMet}$. Various 70S initiation complexes using either f-[^{3}H]Met-$tRNA^{fMet}$ (for dipeptide yield and competition experiments) or fMet-(Cy3)$tRNA^{fMet}$ (for smFRET experiments) were prepared in which the sequence of the second codon was varied

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between GCA (coding for Ala) UUC (coding for Phe) or AAA (coding for Lys). 70S initiation complexes for dipeptide yield and competition experiments were used without further purification whereas those used for smFRET experiments were purified by sucrose density ultracentrifugation in Tris-polymix buffer adjusted to 20 mM $Mg(OAc)_2$.[1]

Ternary complex formation

EF-Tu(GTP)aminoacyl-tRNA ternary complexes were formed by incubating ~2 μM charged tRNA, 12 μM EF-Tu, 9.0 μM EF-Ts, 1.0 mM GTP, 2.5 mM phosphoenolpyruvate, and 2.3 units/mL pyruvate kinase in Tris-polymix buffer at 37 °C for 1 min.

The ternary complexes used in these experiments were prepared with EF-Tu in a six-fold excess over aa-tRNA such that at our experimental concentration, essentially all aa-tRNA was bound to EF-Tu(GTP), even for misacylated tRNAs with impaired EF-Tu affinities. Likewise, the concentration of aa-tRNA, presumably almost entirely bound in EF-Tu(GTP)aa-tRNA ternary complexes, was in a two-fold excess over initiation complex to ensure that initiation complex was limiting. No elongation factor G, class I or II release factors, or ribosome recycling factor was present in the translation reactions; thus, translation reactions are expected to be single turnover under our experimental conditions. After quenching, f-[^{3}H]Met amino acid was separated from f-[^{3}H]Met-X dipeptide by analytical high performance liquid chromatography (HPLC).

smFRET Analysis

Analogous smFRET experiments, using correctly acylated, natural aa-tRNA and initiation complexes programmed with cognate, near-cognate, or non-cognate A-site codons, provided the basis for the current experimental design[6]. Using our highly purified *in vitro* translation system[1], ribosomes were enzymatically initiated onto an mRNA carrying a biotin at the 5'-end, thus placing an AUG codon and the corresponding Cy3 donor fluorophore-labeled fMet-(Cy3)$tRNA^{fMet}$ into the P site and a UUC codon cognate for $tRNA^{Phe}$ into the empty A site[6-8]. Initiation complexes were purified by sucrose density gradient ultracentrifugation and subsequently immobilized onto the surface of a streptavidin-derivatized quartz flow cell[1]. Surface-immobilized initiation complexes carrying P-site fMet-(Cy3)$tRNA^{fMet}$ could then be imaged using a laboratory-built, prism-based, total internal reflection fluorescence microscope as previously described[1]. EF-Tu(GTP)aa-tRNA ternary complexes, prepared using (Cy5)$tRNA^{Phe}$ correctly acylated with Phe or misacylated with Ala or Lys were then stopped-flow delivered to the surface-immobilized initiation complexes. Real-time smFRET *versus* time trajectories of aa-tRNA delivery, selection, and accommodation into the A site of surface-immobilized initiation complexes were then recorded with a 100 msec $frame^{-1}$ time resolution (Fig 3B and C).

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Preparation of aa-tRNAs

The misacylated tRNAs were synthesized using a general ribozyme, the dFX "Flexizyme," recently reported by the Suga group[4]. Significantly, the Flexizyme allows misacylation of dinitrobenzyl ester activated amino acids onto natural, post-transcriptionally modified tRNAs purified from *Escherichia coli*, rather than misacylation *via* ligation of a chemically aminoacylated pdCpA to a truncated tRNA produced by run-off transcription and thus lacking post-transcriptional modifications[12,13]. The tRNA component of our matrix was thus composed of either commercially available amino acid-specific tRNAs over-expressed and purified from *E. coli* ($tRNA^{Phe}$ and $tRNA^{Lys}$, Sigma) or were otherwise purified from a commercially available *E. coli.* total tRNA pool ($tRNA^{Ala}$, Sigma) as described below (Supplementary Fig 1). The dinitrobenzyl ester activated amino acids for aminoacylation by the Flexizyme were synthesized using standard methods, as reported[4] (Supplementary figures 2 and 3). In all cases, optimization of the Flexizyme reaction conditions yielded tRNA aminoacylation efficiencies of ~50%, as determined by thin layer chromatographic separation of [^{32}P]-AMP (**8**) from aminoacyl-[^{32}P]-AMP (**9, 10**) after digestion of the Flexizyme-aminoacylated 3'-[^{32}P]-labeled tRNA with P1 nuclease (Supplementary Fig 4).

Purification of $tRNA^{Ala}$

Purification of $tRNA^{Ala}$ was accomplished in three steps - aminoacylation, formation of ternary complexes, and column chromatography based on previously described protocols[9,10]. Briefly, those steps were performed as follows:

The total tRNA pool (Roche) was first deacylated by dissolving 10 mg in 200 μL 0.2 mM sodium borate, pH 8 and incubating for 1 hour at 37 °C. This solution was subsequently desalted over a Bio-gel P-6 spin column (Bio-Rad, Inc). To this solution, NaOAc, pH 5 was added to a final concentration of 0.3 M and the tRNA was precipitated with ethanol.

Aminoacylation of $tRNA^{Ala}$ was performed at 37 °C for 10 minutes under the following conditions: 20 mM Tris-HCl, pH 7.5, 20 mM KCl, 10 mM $MgCl_2$, 5 mM DTT, 6.25 mM ATP, 800 A_{260} units of tRNA pool, 13 mM alanine, 2 μM alanyl-tRNA synthetase in a total volume of 2 mL. Following incubation, the reactions were quenched with 200 μL 3M NaOAc, pH 5 and precipitated at -80 °C with 2.7 volumes of 100% ethanol. Precipitated alanine-$tRNA^{Ala}$ was resuspended in 400 μL 10 mM KOAc, pH 5 immediately before use.

GTP exchange was accomplished by incubating 90 μM six-histidine tagged EF-Tu(GDP) at 37°C for 10 minutes in the presence of 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 150 mM NH_4Cl, 5 mM 2-mercaptoethanol, 5 mM phosphoenolpyruvate, 75 units/mL pyruvate kinase, 1 mM GTP, 9 μM EF-Ts in a total volume of 3.6 mL. Ternary complex was subsequently formed by adding 400 μL of the product from the aminoacylation reaction and incubating for a further 10 minutes on ice.

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The ternary complex solution was then added to an equal volume of Ni^{2+}-nitrilotriacetic acid-agarose resin (Qiagen) pre-equilibrated with 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 150 mM NH_4Cl, 10 mM 2-mercaptoethanol and 250 μM GTP which was then incubated at 4°C for 1 hour with agitation.

Two main buffers were used for the column chromatography. Buffer C which consisted of 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 150 mM NH_4Cl, 10 mM 2-mercaptoethanol; and Buffer TM which was 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1M NaCl, 10 mM 2-mercaptoethanol.

The resin was washed with 5 column volumes Buffer C with 1 mM GTP, and then with 15 column volumes of Buffer C with 250 μM GTP. The purified alanine-$tRNA^{Ala}$ was eluted with 5x 1 column volume of Buffer TM. The purified $tRNA^{Ala}$ was deacylated in 0.2 mM sodium borate, pH 9 for 30 minutes, and the RNA was recovered by ethanol precipitation. Deacylated $tRNA^{Ala}$ was further purified by FPLC using a TSK-phenyl 5PW column (Tosoh Bioscience) and a gradient that ran from 1.7M NH_4SO_4/10 mM NH_4OAc, pH 6.2 to 10 mM NH_4OAc/10% Methanol over 25 column volumes.

Finally, the purified alanine tRNA was tested by aminoacylation with tritium labeled alanine using the alanyl-tRNA synthetase. See supplementary figure 1.

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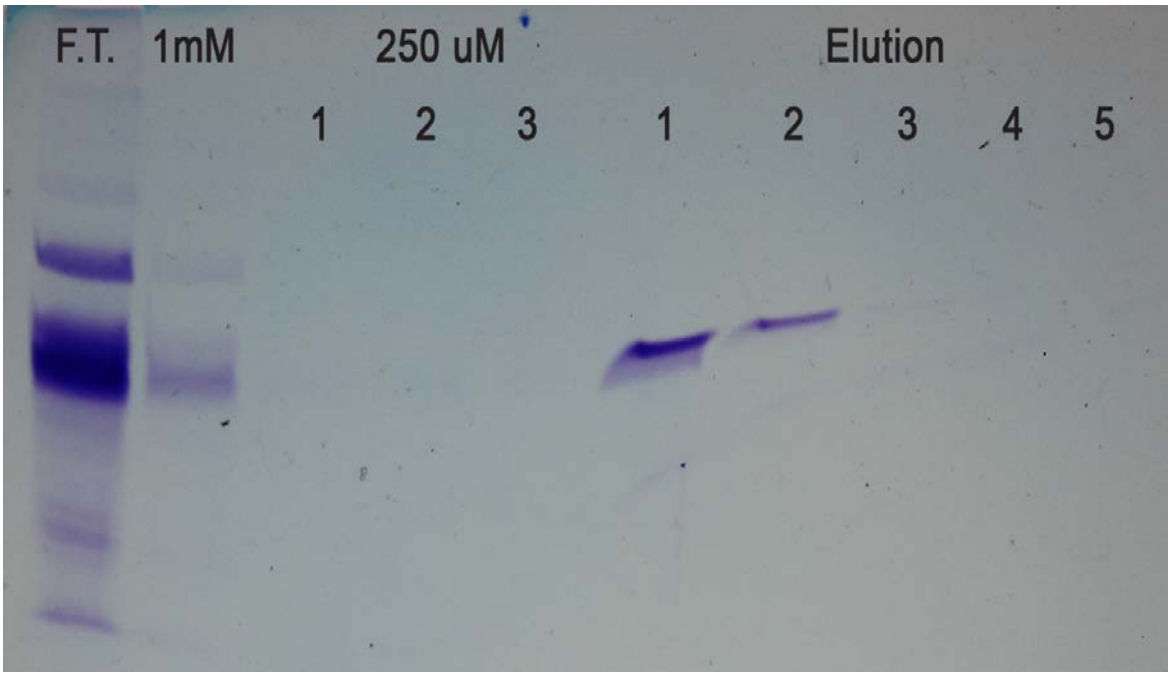

Supplementary Figure 1. Summary of Ni-NTA column purification of $tRNA^{Ala}$. Lane 1, flowthrough. Lane 2, wash using buffer C with 1 mM GTP. Lane 3-5, washes using buffer C with 250 μM GTP. Lanes 6-10, elutions under high salt conditions with buffer TM.

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Synthesis of Dinitrobenzyl Ester Activated Amino Acids

The amino acid 3,5-dinitrobenzyl ester substrates were synthesized from the corresponding Boc protected amino acids (N_α-Boc-L-Alanine, 10 (Novabiochem, 98% purity) and N_α,N_ε-*bis*-Boc-L-Lysine, 11 (Novabiochem, 98% purity)). ^{1}H NMR spectra were recorded on a Bruker DPX-400 (400MHz) spectrometer and are reported in ppm using MeOD (Cambridge Isotope Laboratories) as the solvent. NMR data are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet; dd, doublet of doublets.

Representative procedure for the synthesis of DBE substrates (alanine-3,5-dinitrobenzyl ester): A mixture of α-*N*-Boc-alanine (300 mg, 1.58 mmol), triethylamine (270 mg, 2.65 mmol) and 3,5-dinitrobenzyl chloride (286 mg, 1.32 mmol) in 2.0 mL of dimethylformamide was stirred at room temperature overnight. Diethylether (36 mL) was added and the solution was washed with 0.5 M HCl (12 mL x 3), 4 % $NaHCO_3$ (12 mL x 3) and brine (20 mL x1), and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was dissolved in 8 mL of 4 M HCl/ethyl acetate and stirred for 20 min at room temperature. The solution was concentrated *in vacuo* and the remaining HCl was removed by adding diethylether (8 mL) and concentrating *in vacuo* three times. The product was precipitated by the addition of diethylether (10:1 v/v) to methanol, and the precipitate was filtered in 30% overall yield (128 mg, 0.474 mmol).

Alanine-3,5-Dinitrobenzyl Ester, (**11**)*.* Clear solid (30% yield): ^{1}H NMR (400MHz, MeOD) δ 8.94(t, *J* = 2, 1H, Ar-**H**), 8.64(d, *J* = 2, 2H, Ar-**H**), 5.47(s, 2H, Ar-**CH_2**), 4.21(q, *J* = 7, 1H, CH_2-C**H**), 1.55(d, *J* = 3, 3H, CH-C**H_3**) (See supplementary Fig 2)
LRMS (FAB+), *m/z* calculated from $C_{10}H_{11}N_3O_6$ 269, found ($M+H^+$) 270.

Lysine-3,5-Dinitrobenzyl Ester (**12**)*.* Yellow solid (35% yield): ^{1}H NMR (400MHz, MeOD) δ 8.94(t, *J* = 2, 1H, Ar-**H**), 8.65(d, *J* = 2, 2H, Ar-**H**), 5.49(s, 2H, Ar-**CH_2**), 4.18(t, *J* = 6, 1H, CH_2-C**H**), 2.91(t, *J* = 7, 2H, NH_2-C**H_2**), 2.09-1.884 (m, 2H, CH-C**H_2**), 1.69(t, *J* = 7, 2H, CH_2-C**H_2**), 1.54(m, 2H) (See supplementary Fig 3)
LRMS (FAB+), m/z calculated from $C_{13}H_{18}N_4O_6$ 326, found ($M+H^+$) 327.

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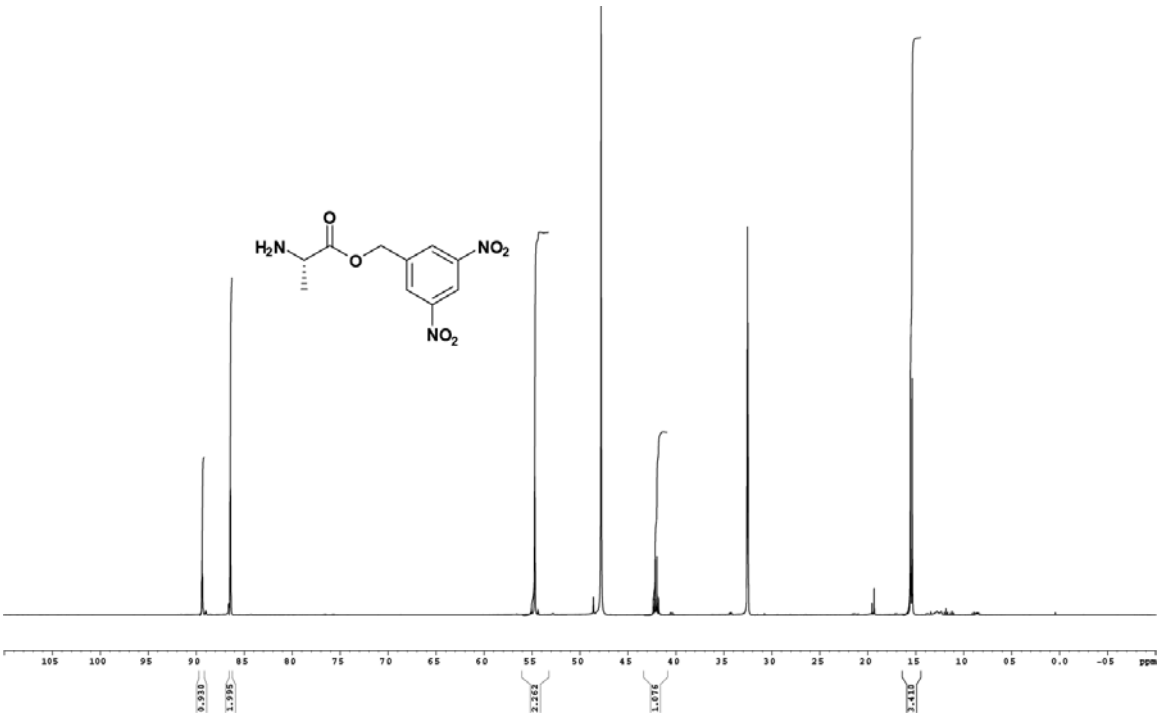

Supplementary Figure 2. ^{1}H NMR of Alanine-3,5-Dinitrobenzyl Ester

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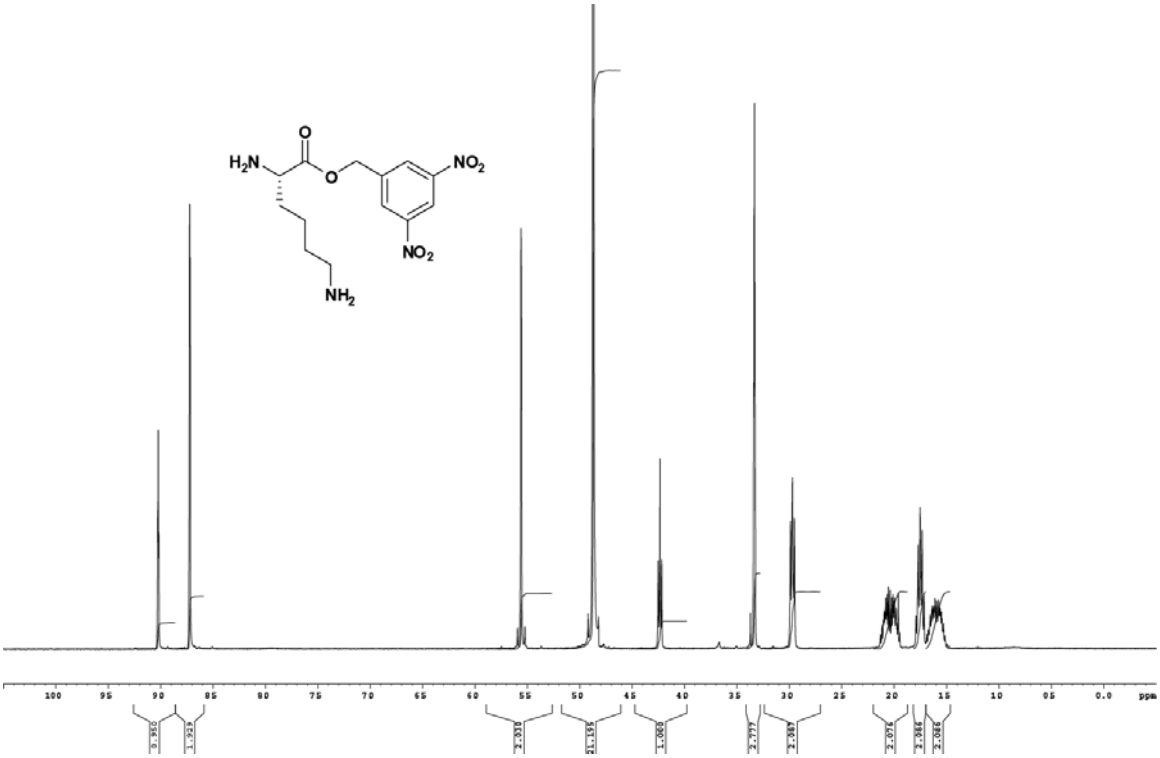

Supplementary Figure 3. ^{1}H NMR of Lysine-3,5-Dinitrobenzyl Ester

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Aminoacylation with the dFx Flexizyme ribozyme

The aminoacylation reactions were carried out at 20 μM tRNA, 20 μM dFx, 5 mM aminoacid-DBE substrate in 0.1 M Hepes-K, pH 7.5, 0.1 M KCl, 600 mM $MgCl_2$, 20% DMSO. The reactions were incubated on ice for 30 minutes when charging tRNA with alanine, and for 2 hours when charging with lysine. Reactions were subsequently quenched by the addition 3x volumes of 600 mM NaOAc, pH 5 to achieve a final concentration of 450 mM. The aa-tRNA was recovered by ethanol precipitation.

To estimate the aminoacylation efficiencies, analytical scale aminoacylations were performed concurrently under the same conditions outlined above with the exception that [^{32}P]-labeled tRNA was used[11]. After acidic quenching and ethanol precipitation, the aa-tRNAs were resuspended in 10 mM KOAc, pH 5 and digested with nuclease P1 (Sigma, Inc) for 10 minutes at room temperature[11]. Separation of [^{32}P]-AMP from aminoacyl-[^{32}P]-AMP was subsequently achieved by thin-layer chromatography on PEI-cellulose plates (EMD Chemicals, Inc) under acidic conditions (100 mM NH_4Cl, 10% acetic acid). Following separation, the TLCs were exposed on a storage phosphor screen and quantitated with a phosphoimager. (Supplementary Fig 4) Charging efficiencies were calculated as the percentage of aminoacyl-[^{32}P]-AMP out of total [^{32}P]-AMP. Nucleotidyl transferase for [^{32}P] labeling of tRNA 3'-ends was purified from an overexpressing strain kindly provided by Prof. Ya-Ming Hou and Dr. Marcel Dupasquier.

The measured aminoacylation efficiencies were then applied to the total tRNA concentration, after correcting for the presence of the Flexizyme, to obtain the final aa-tRNA concentrations for translation reactions. The Flexizyme-aminoacylated tRNAs were not further purified after aminoacylation; at the Mg^{2+} concentrations used for the translation reactions (3.5 mM), the Flexizyme should not perturb the translation reaction[14]. Because deacylated tRNA is well established to be a poor substrate for EF-Tu(GTP) deacylated tRNA should not affect the translation reaction. These general methods for synthesis and characterization of the aa-tRNAs provided ready access to the misacylated tRNAs required for these studies.

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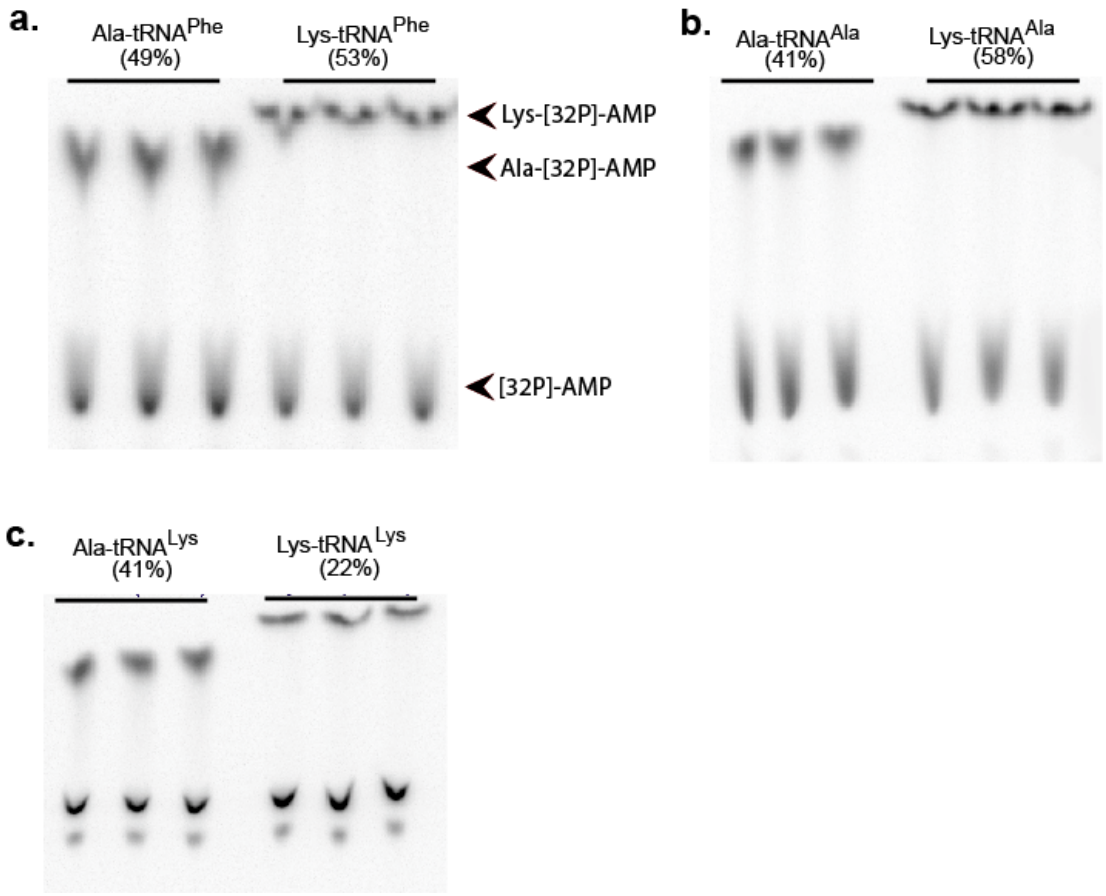

Supplementary Figure 4. TLC analyses of aminoacylation efficiency. The aa-tRNA species analyzed and the calculated charging efficiencies are indicated at the top of the TLC. Each sample was spotted in triplicate. A) Ala-tRNA^{Phe} and Lys-tRNA^{Phe} were charged to 49% and 53% respectively. B) Ala-tRNA^{Ala} and Lys-tRNA^{Ala} were charged to 41% and 58% respectively. C) tRNA^{Lys} was charged with Lys and Ala to 22% and 41% respectively.

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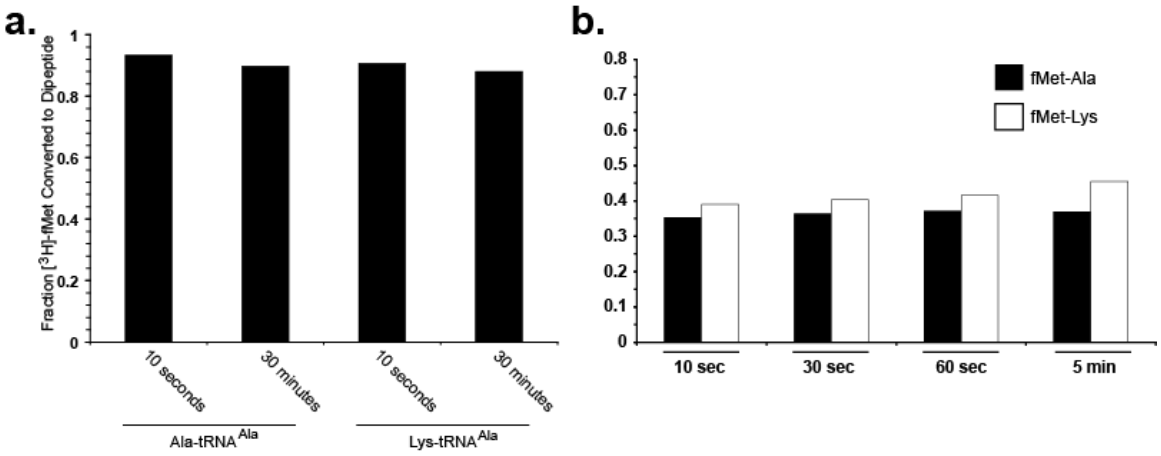
Supplementary Results



Supplementary Figure 5. Sample dipeptide yield and competition translation time course data. A) Fraction of fMet converted to dipeptide for Ala-tRNA^{Ala} and Lys-tRNA^{Ala} at 10 seconds and 30 minutes in dipeptide synthesis yield translations. B) Fraction of fMet converted to fMet-Ala and fMet-Lys with increasing time in a competition translation involving Ala-tRNA^{Ala} and Lys-tRNA^{Ala}. These data represent the average of two translations.

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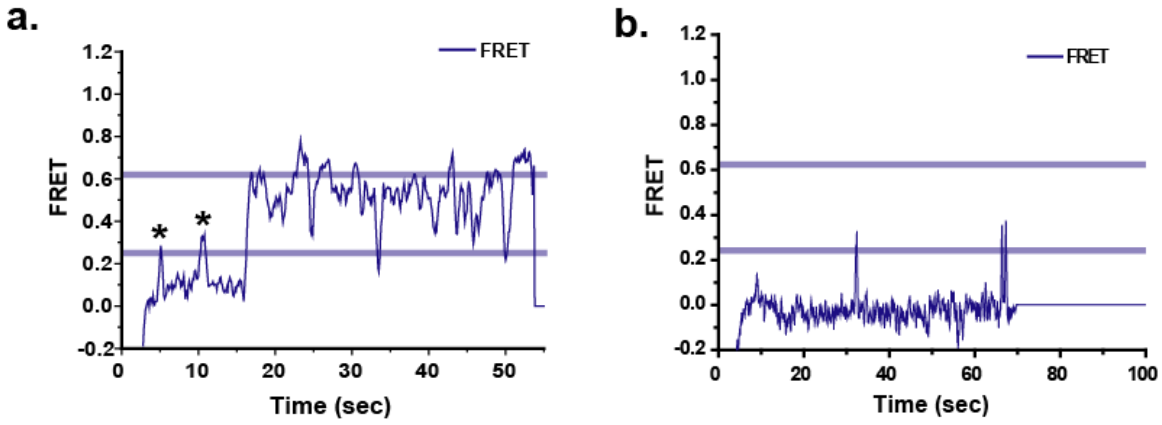

Supplementary Figure 6. Sample FRET traces. The smFRET traces were analyzed using two FRET boundaries at 0.25 and 0.62 FRET (blue lines), which define three FRET windows, ≤0.24 FRET, 0.25-0.61 FRET and ≥0.62 FRET. Using these criteria we categorize smFRET traces as either productive (A) or non-productive (B). Productive traces (A) display productive binding events, which are those events that enter the 0.25-0.61 FRET window and rapidly cross into the ≥0.62 FRET window (i.e. progress to full accommodation/peptide bond formation) during the observation period. Non-productive traces (B) display only non-productive A-site sampling events, which are defined as events that enter the 0.25-0.61 FRET window but rapidly return to the ≤0.24 FRET window (i.e. dissociate) without crossing into the ≥0.62 FRET window. While productive traces may exhibit several A-site sampling events prior to a productive binding event (shown in panel A, asterisks), non-productive traces never exhibit productive binding events (the smFRET signal never crosses into the ≥0.62 FRET window.

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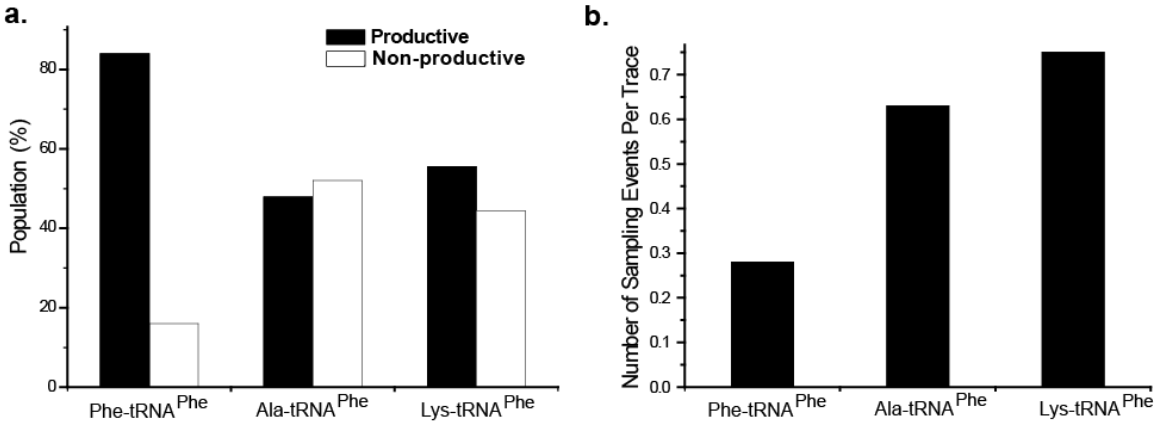

Supplementary Figure 7. Subpopulation analysis for smFRET experiments. (A) The percentage of traces that ultimately exhibited productive binding events (productive traces, solid bars) and the percentage that exhibited A-site sampling events only (non-productive traces, open bars) are indicated here. Of the 200 Phe-(Cy5)tRNA^{Phe} traces analyzed, 84% ultimately exhibited productive events and 16% of the traces were non-productive, exhibiting only A-site sampling events. Of the 192 Ala-(Cy5)tRNA^{Phe} traces analyzed, 48% were productive and 52% were non-productive; and of the 153 Lys-(Cy5)tRNA^{Phe} traces analyzed, 56% and 44% were productive and non-productive respectively. (B) The frequency of sampling events for correctly and misacylated (Cy5)tRNA^{Phe} expressed as the number of sampling events per trace. These are the combined averages from productive and non-productive traces. The recorded frequencies were 0.28, 0.63 and 0.75 for Phe, Ala and Lys on (Cy5)tRNA^{Phe} respectively.

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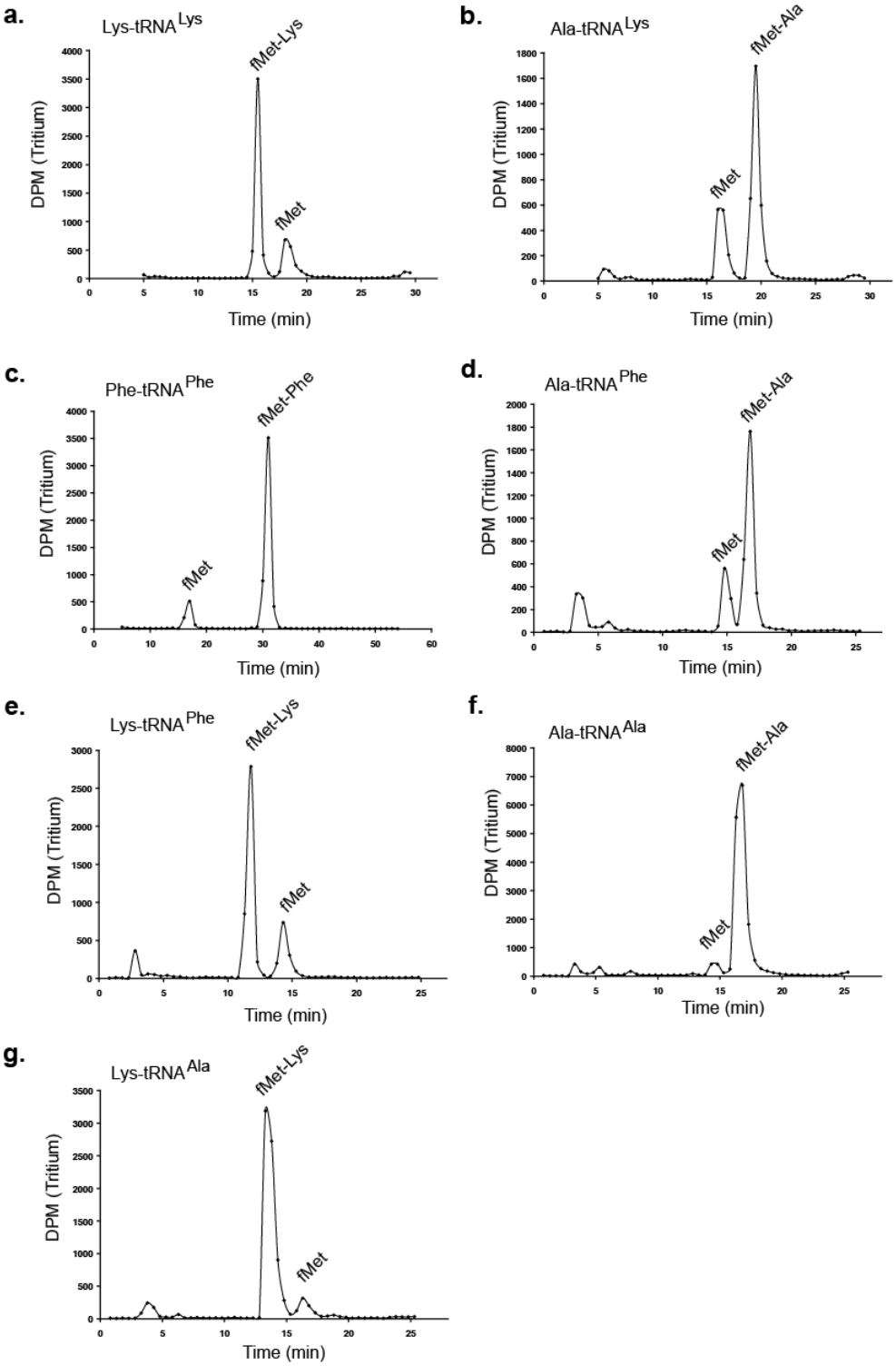

Supplementary Figure 8. Representative scintillation traces for dipeptide yield translations. All dipeptide yield reactions were allowed to proceed for 10 sec and were quenched by base hydrolysis. The dipeptides were separated from unreacted f-[^{3}H]Met by reversed-phase HPLC (C18 column, Vydac) over a gradient that progressed from

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1% acetonitrile in 0.1% TFA to 1.8% acetonitrile in 18 minutes, and then to 100% acetonitrile in 20 minutes. Fractions were collected in either 30 sec or 1 min increments, and were subsequently quantified by scintillation counting. Authentic markers were used to verify retention times. Representative scintillation traces of dipeptide yield experiments involving Lys-tRNA^{Lys}, Ala-tRNA^{Lys}, Phe-tRNA^{Phe}, Ala-tRNA^{Phe}, Lys-tRNA^{Phe}, Ala-tRNA^{Ala} and Lys-tRNA^{Ala} are shown in panels A-G respectively.

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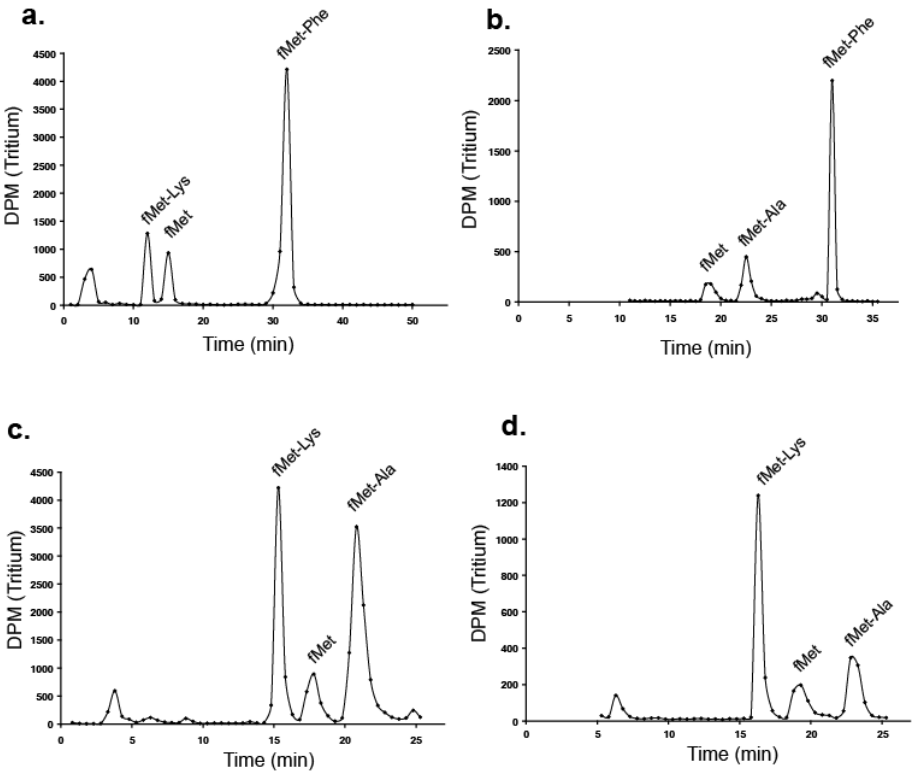

Supplementary Figure 9. Representative scintillation traces for competition translations. Competition translations were worked up and analyzed as described for dipeptide yield translations. Representative traces for Phe-tRNA^{Phe} vs Lys-tRNA^{Phe}; Phe-tRNA^{Phe} vs Ala-tRNA^{Phe}; Ala-tRNA^{Ala} vs Lys-tRNA^{Ala} and Lys-tRNA^{Lys} vs Ala-tRNA^{Lys} are shown in panels A-D respectively.

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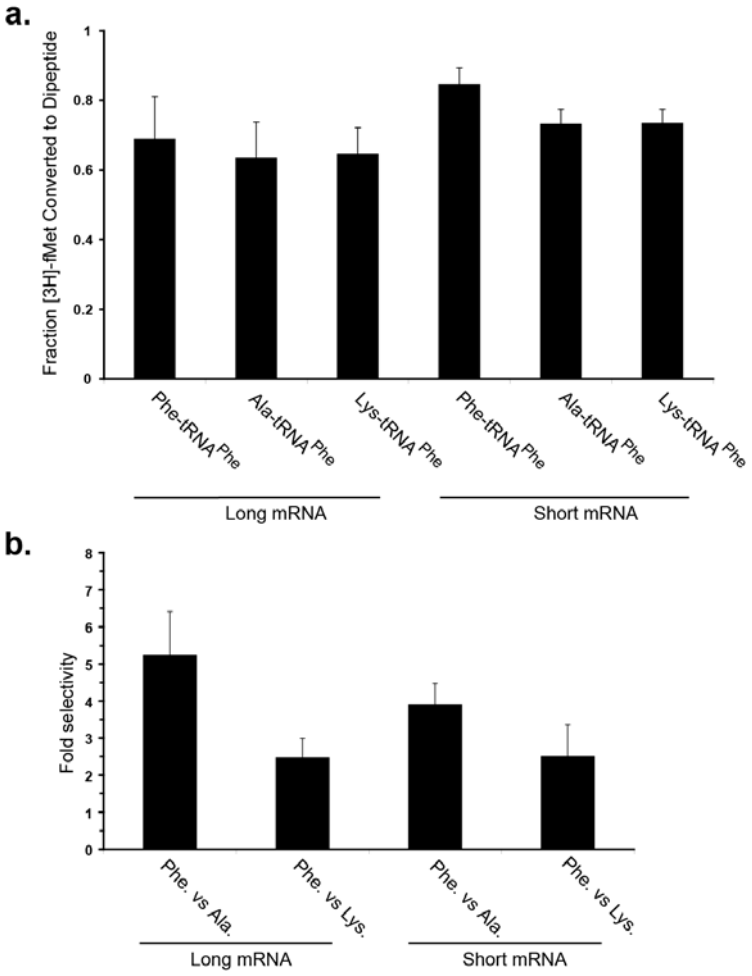

Supplementary Figure 10. mRNA length does not alter selection. A) Dipeptide yield experiments comparing a long mRNA encoding the first 20 codons of the T4 gene product 32, and a shorter mRNA encoding fMet-X-Glu where X encodes either Phe, Ala or Lys. In both cases the dipeptide yield of the misacylated tRNAs is within error of the yield from the correctly acylated tRNA. B) Fold selectivity from competition experiments calculated as the ratio of products from correctly acylated relative to misacylated tRNAs. 3.7- and 2.2-fold decreases in selection efficiency were observed for Ala-tRNA^{Phe} and Lys-tRNA^{Phe} respectively *versus* Phe-tRNA^{Phe} using the short mRNA. The decreases in selection efficiency observed with the longer mRNA were 5- and 2.5 –fold for Ala-tRNA^{Phe} and Lys-tRNA^{Phe} respectively *versus* Phe-tRNA^{Phe}**.**

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Supplementary Tables

Supplementary Table 1. Fitting parameters from smFRET data analysis

	Initial Binding			Accommodation		
aa-tRNA	A_1	τ_1 (sec)	k_{bind} ($x10^7$ M^{-1} sec^{-1})	A_1	τ_1 (sec)	k_{acc} (sec-1)
Phe-(Cy5)$tRNA^{Phe}$	156	7.89	5.63 ± 0.14	100	0.2	5.0 ± 1.2
Ala-(Cy5)$tRNA^{Phe}$	124	22.13	2.01 ± 0.007	100	0.17	6.3 ± 2.7
Lys-(Cy5)$tRNA^{Phe}$	130	14.88	2.99 ± 0.10	96	0.17	5.9 ± 3.8

Initial binding curves were best described by exponential growth curves of the form $A_1(1-\exp(-t/\tau_1))$; $k_{bind}=1/(\tau_1*[\text{aa-tRNA}])$. Dwell times were best described by single exponential decays of the form $A_1(\exp(-t/\tau_1))$; $k_{bind}=1/\tau_1$.

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