Supplemental Information

Hyperaccurate and error-prone ribosomes exploit distinct mechanisms during tRNA selection

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Supplemental Data:

Figure S1 Kinetic parameters for cognate ternary complexes

(A) Determination of apparent k_2 and relative amount of aa-tRNA that goes through the initial selection by monitoring fluorescence changes in Leu-tRNA$_2^{Phe}$ (Prf16/17/20). Ternary complex containing labeled aa-tRNA (0.15 μM after mixing) was rapidly mixed with ICs (1 μM after mixing) displaying the Phe UUC codon in the A site. Each phase of the signal was fit to single-exponential kinetics (smooth lines) individually.

(B) Determination of k_{-2}. The time-courses were obtained by first generating codon-recognition complexes containing the GTPase-deficient EFTu H84A (0.3 μM after mixing) and then rapidly mixing it with tenfold excess of ternary complex containing unlabeled Phe-tRNA^{Phe}.

(C) Time-courses of GTP hydrolysis on the ternary complex EFTu.GTP.Phe-tRNA^{Phe} with 1 μM ICs.

(D) Time-courses of dipeptide formation. Ternary complex was added to a final concentration of 0.5 μM. As a result the maximal expected end-point is 0.5.

Figure S2 The effects of the *ram* and *restrictive* mutations on the proofreading phase as studied using Glu-tRNA^{Glu}

In these sets of experiments, initiation complexes were programmed with mRNAs that presented either the codon GAA (cognate for $tRNA^{Glu}$, and labeled cognate IC in the figure) or GAU (near-cognate for $tRNA^{Glu}$, and labeled near-cognate IC in the figure) in the A site. The $tRNA^{Glu}$ was used because it was found to readily misread the near-cognate codon GAU. $tRNA^{Glu}$ misreads the near-cognate GAU codon with an observed rates of peptide-bond formation (~1.2 s^{-1}) and end-point (~0.25) values that are almost identical on both WT and ram ribosomes. Also similar to the earlier observed pattern, the *restrictive* ribosome reduced this error rate by decreasing the end-point to 0.04, while keeping the rate similar to the WT (1.3 s^{-1}). Reactions conducted with cognate ICs display similar rates and endpoints among the three ribosomes.

Figure S1

Zaher & Green, Supplemental Figure 1

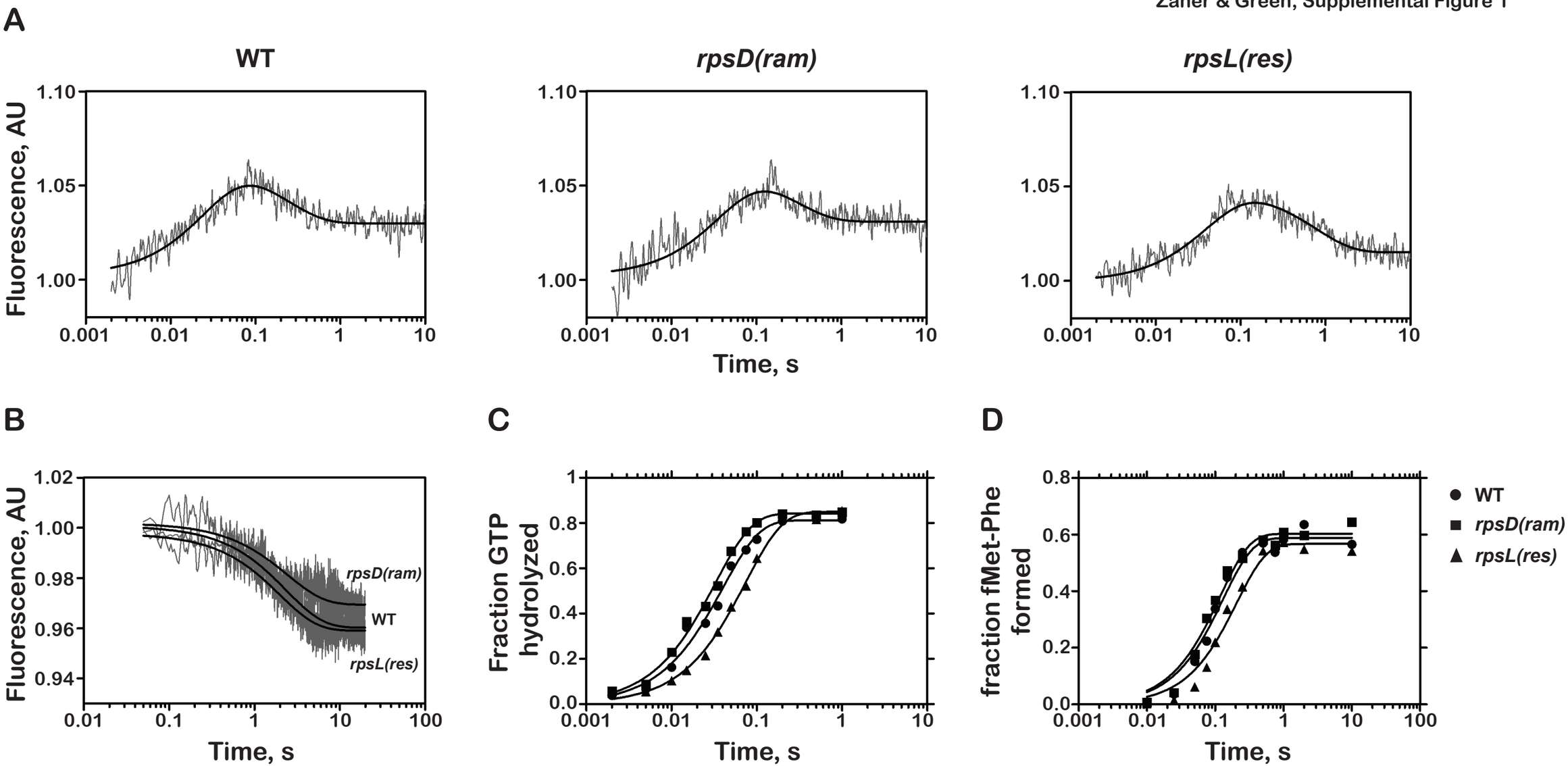

Figure S2

Zaher & Green, Supplemental Figure 2