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Supporting Material

Codon-Dependent tRNA Fluctuations Monitored with Fluorescence Polarization

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Instrumental setup for the FRET acceptor emission polarization measurements

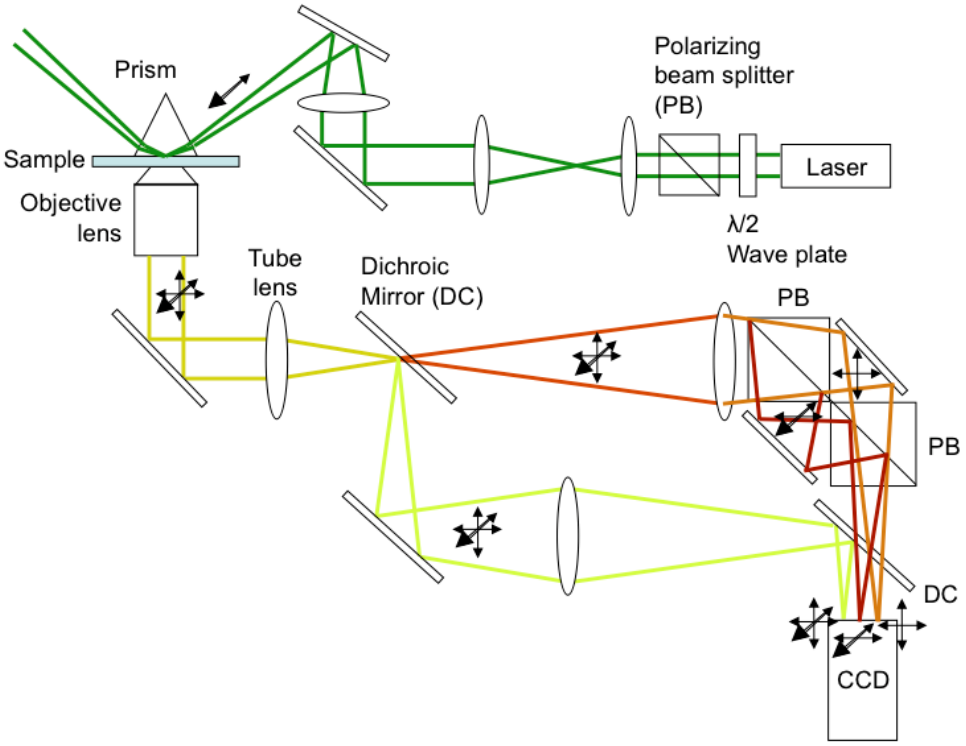

Fig. S1. Instrumental setup for the FRET acceptor emission polarization measurements. 532nm laser beam is parallel polarized to the optical axis. The fluorescence emission is divided into two spectral regions (550-645nm and 645-750nm). The 645-750nm emission is further divided into two polarization components. An electron multiplying charge coupled device (EMCCD) chip is divided into three different regions to separately image fluorescence from 550-645nm, 645-750nm parallel polarization component and 645-750nm perpendicular polarization component. FRET efficiency is calculated as $I_{cy5,\parallel} + I_{cy5,\perp} / (I_{cy3} + I_{cy5,\parallel} + I_{cy5,\perp})$ and fluorescence polarization is calculated as $(I_{cy5,\parallel} - I_{cy5,\perp})/(I_{cy5,\parallel} + I_{cy5,\perp})$.

GDPNP stalled state of the ribosome complexes

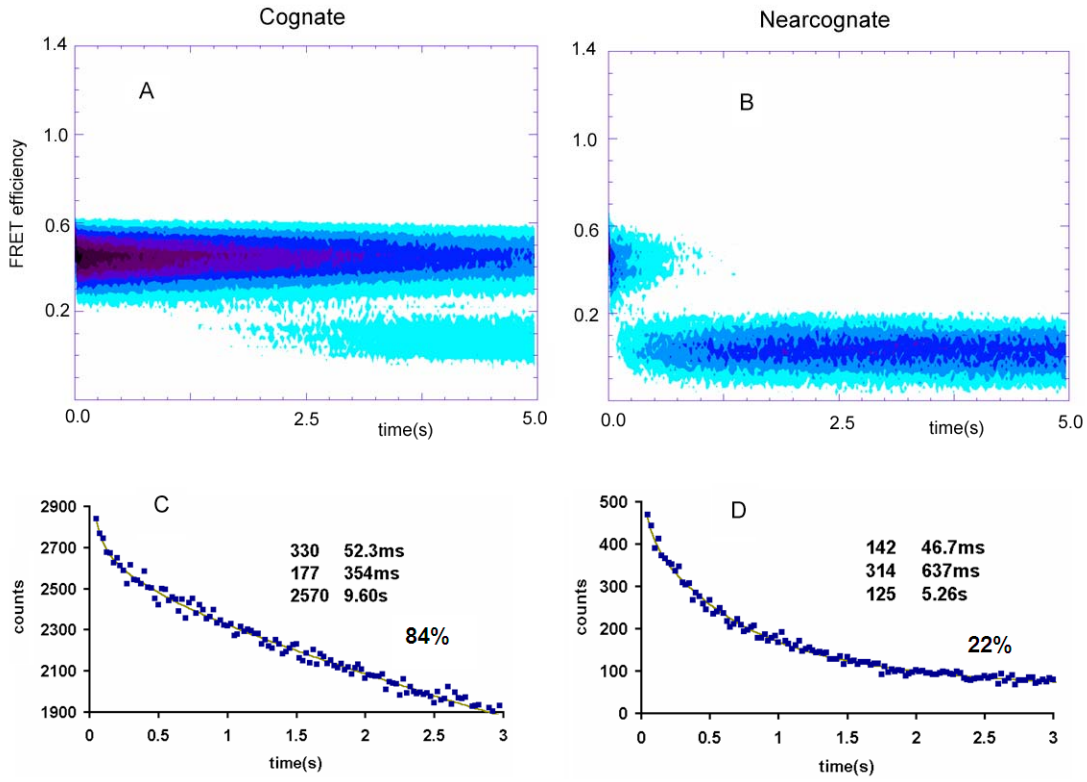

Fig. S2. GDPNP stalled state of the cognate and nearcognate ribosome complexes. (A~B) the post-synchronized plot of the mid-FRET state (GTPase activated state) of the cognate and nearcognate complexes. Post-synchronization is done at FRET efficiency of 0.42. (C~D) exponential fit of the GTPase activated state (FRET 0.42~0.62) lifetimes. Three exponential components are evident. The shortest component is noise included during post-sync and the sub-sec component is likely due to dye blinking. The longest component is GDPNP stalled state. Both complexes show GDPNP stalled state that lasts at least several seconds. The short excursions to the high-FRET state cannot be seen in these ensemble-averaged plots.

Fluorescence Polarization Histograms

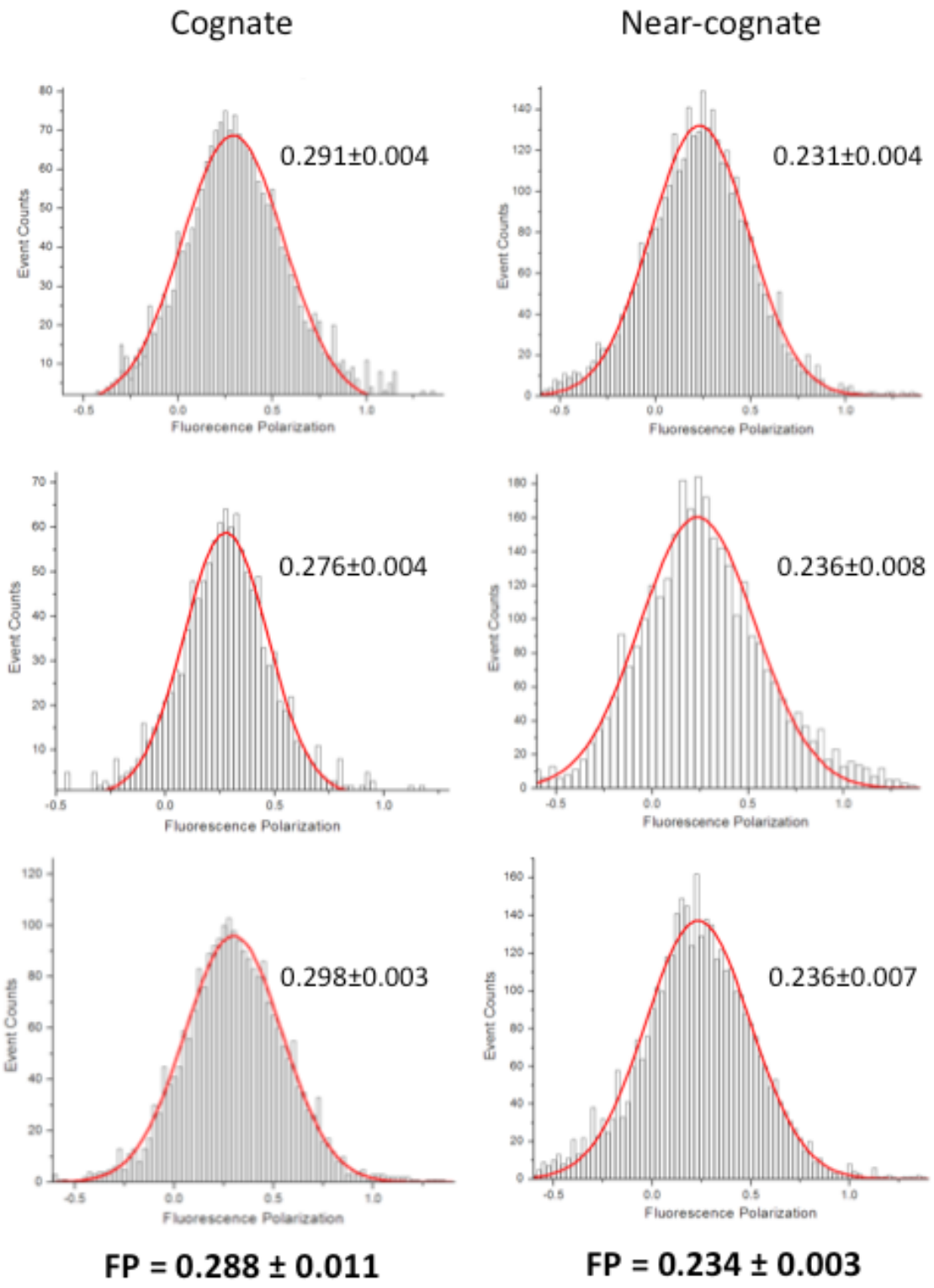

Fig. S3. Fluorescence polarization of Cy5 labeled at phe-tRNA^{phe} in cognate and near-cognate complexes

Fluorescence polarization of FRET acceptor

Fluorescence polarization decay of an initially parallel-aligned fluorescence emission dipole "a" can be expressed as below assuming that the rotational diffusion follows a single exponential decay with a rate constant k_{Ra}:

$$P(t) = \frac{I_{a,\parallel}(t) - I_{a,\perp}(t)}{I_{a,\parallel}(t) + I_{a,\perp}(t)} = e^{-k_{Ra}t}$$

The denominator of the equation should be substituted with $I_{a,\parallel}(t)+2I_{a,\perp}(t)$ for anisotropy, which is a more appropriate measure of polarization in a typical fluorimeter setup.

One more assumption in the above equation is that the fluorescence decay is much slower than the rotational diffusion of the dipole. With a slowly diffusing dipole, however, this assumption may not be valid. For a fluorescence emission dipole with an emission decay rate of k_a, the probability of emitting a photon at time t (t=0 is defined as the moment of excitation) is defined by $k_a e^{-k_a t}$. Therefore, the observable fluorescence polarization at time t can be corrected as follows for a slowly diffusing dipole.

$$P(t) = k_a e^{-k_a t} e^{-k_{Ra} t}$$

Integrating the equation yields the total fluorescence polarization from a dipole collected over a long period of time during which the dipole experiences many excitation/emission cycles.

$$P = \int_0^\infty k_a e^{-k_a t} e^{-k_{Ra} t} dt$$

When the fluorescence is due to FRET, we need to consider the rotational diffusion of the donor and the acceptor during the energy transfer. If the donor diffusion is much slower than the acceptor diffusion, the relative rotational diffusion of the acceptor to the donor can be approximated to the absolute acceptor diffusion with a rate k_{Ra}. The fluorescence decay rate should be replaced with FRET decay rate k_f. Polarized FRET from the donor to the acceptor is then as follows.

$$P_{FRET}(t') = k_f e^{-k_f t'} e^{-k_{Ra} t'}$$

This equation gives the amount of polarized FRET at time t' some of which will be emitted polarized from the acceptor. The total observable polarized emission from the acceptor due to the polarized FRET at time t' is then as follows:

$$P_{acceptor}(t') = k_f e^{-k_f t'} e^{-k_{Ra} t'} \int_0^\infty k_a e^{-k_a t} e^{-k_{Ra} t} dt$$

Integration of the equation over time t' yields the total fluorescence polarization from the acceptor during the entire FRET decay time window.

$$P = \frac{1}{2} \int_0^\infty k_f e^{-k_f t'} e^{-k_{Ra} t'} \int_0^\infty k_a e^{-k_a t} e^{-k_{Ra} t} dt dt' = \frac{1}{2} \frac{k_f}{k_f + k_{Ra}} \cdot \frac{k_a}{k_a + k_{Ra}}$$

The factor of ½ is to incorporate the equation to consider the absorption by dipoles that are not perfectly parallel to the excitation polarization. Effect of FRET between

imperfectly aligned donor-acceptor is ignored, as the resulting excited acceptor will be randomly aligned to the external excitation polarization and consequently can increase or decrease the acceptor emission polarization. Therefore, the resulting average polarization over the signal integration time should not be affected by this factor.

Rate constants analysis

Rate constants in and out of the GTPase activated state (delivered in the form of aa-tRNA/EF-Tu/GDPNP ternary complex) were analyzed by an HMM algorithm(1). Mid-FRET state (0.4~0.5 FRET) with at least 100ms or longer were included in the analysis and shorter ones were discarded in order to obtain the lifetimes of the legitimate GTPase activated state only. At least 50 FRET traces per case were included in the analysis. The stalled GTPase activated state (mid-FRET state) lifetimes are likely underestimated due to the limited photobleaching lifetime of Cy5. Note that, due to the longer signal integration time (35ms) than the lifetime of the low FRET state (0.2~0.3 FRET, ~20ms), the number of detectable states is two (0.4~0.5 and >0.7 FRET for mid and high-FRET, respectively) instead of three (0.2~0.3, 0.4~0.5 and >0.7 FRET)(2,3). The short lifetime of codon recognition complex of a near-cognate in an ensemble measurement (1/17 s)(4) is probably due to the averaged short and long lifetime components of the apparent GTPase activated state(2) all together.

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2. Lee, T.-H., Blanchard, S.C., Kim, H.D., Puglisi, J.D. and Chu, S. (2007) The role of fluctuations in the initial selection of tRNA by the ribosome. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 13661-13664.
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4. Pape, T., Wintermeyer, W. and Rodnina, M. (1999) Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *EMBO J*, **18**, 3800-3807.