Supporting information for

Role of the ribosomal protein L27 at the peptidyl transfer center revealed by single molecule FRET study

Figure S1.

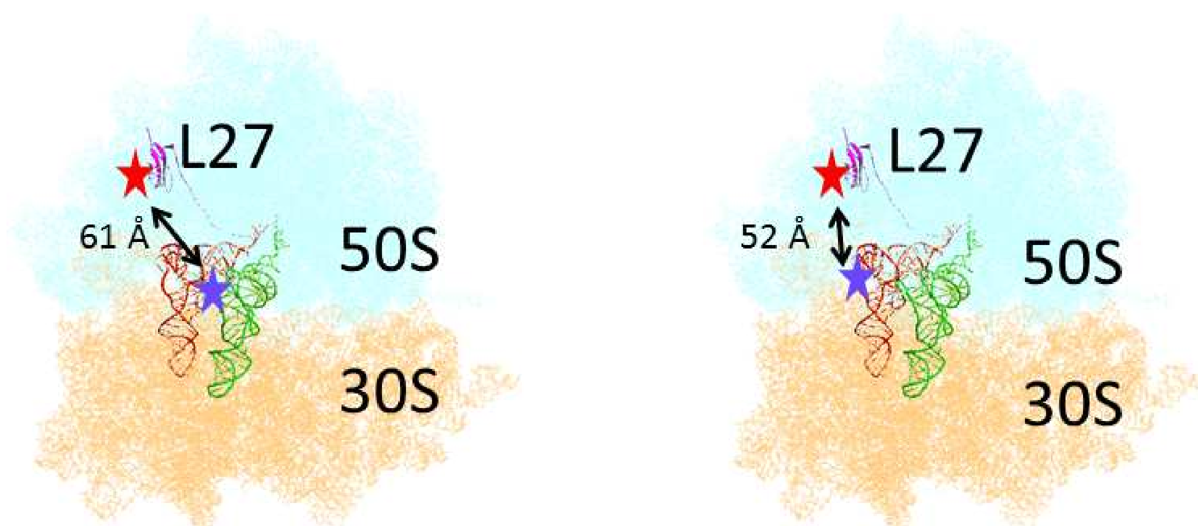

Figure S1. The FRET labeling strategy. The ribosome 50S and 30S subunits are represented in cyan and orange. A- and P-site tRNAs are represented by green and red colors. The distances between the anchors for the Cy5 dye on L27 and the Cy3 Dye on the A- or P-site tRNA are 61 Å and 52 Å respectively. The coordinates are adapted from [1].

Figure S2.

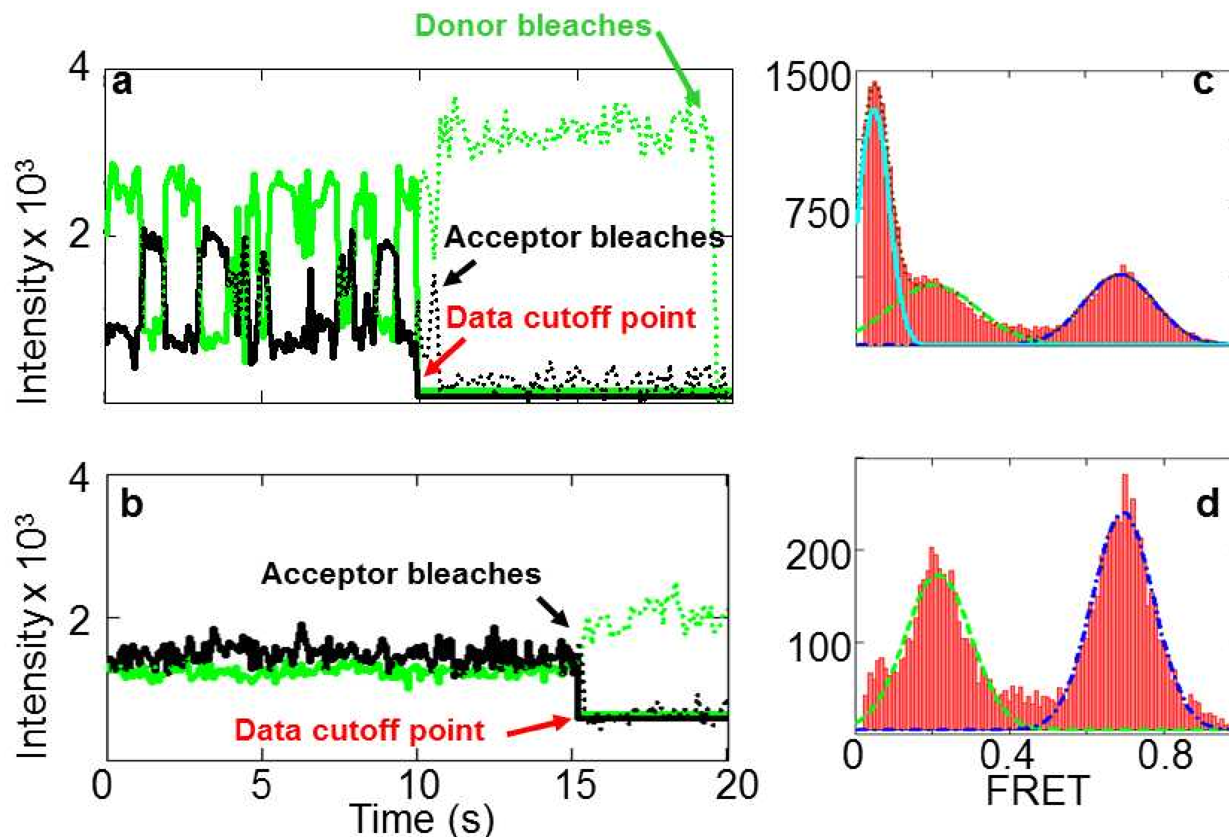

Figure S2. Data selection and processing. One pair of the original raw fluorescence traces from donor (green) and the acceptor (black) is shown. The single molecule signals are selected based on single-step bleaching. Plots (a) and (b) give examples of fluctuating and non-fluctuating signals, respectively. The data are truncated at a few points prior of the bleaching events to remove the artificial FRET signals from donor crosstalk (the solid lines of plots (a) and (b)). The sequential bleaching of the acceptor and the donor, and the data cutoff points are highlighted in (a) and (b). Plots (c) and (d) show the FRET efficiency histograms without and with the removal of the data points after donor bleaching, respectively. Plot (c) is generated from the data points shown by the dashed lines in plots (a) and (b) while plot (d) is generated from the data points shown by the solid lines in plots (a) and (b). Removal of the data points after donor bleaching efficiently removes background noise.

Figure S3.

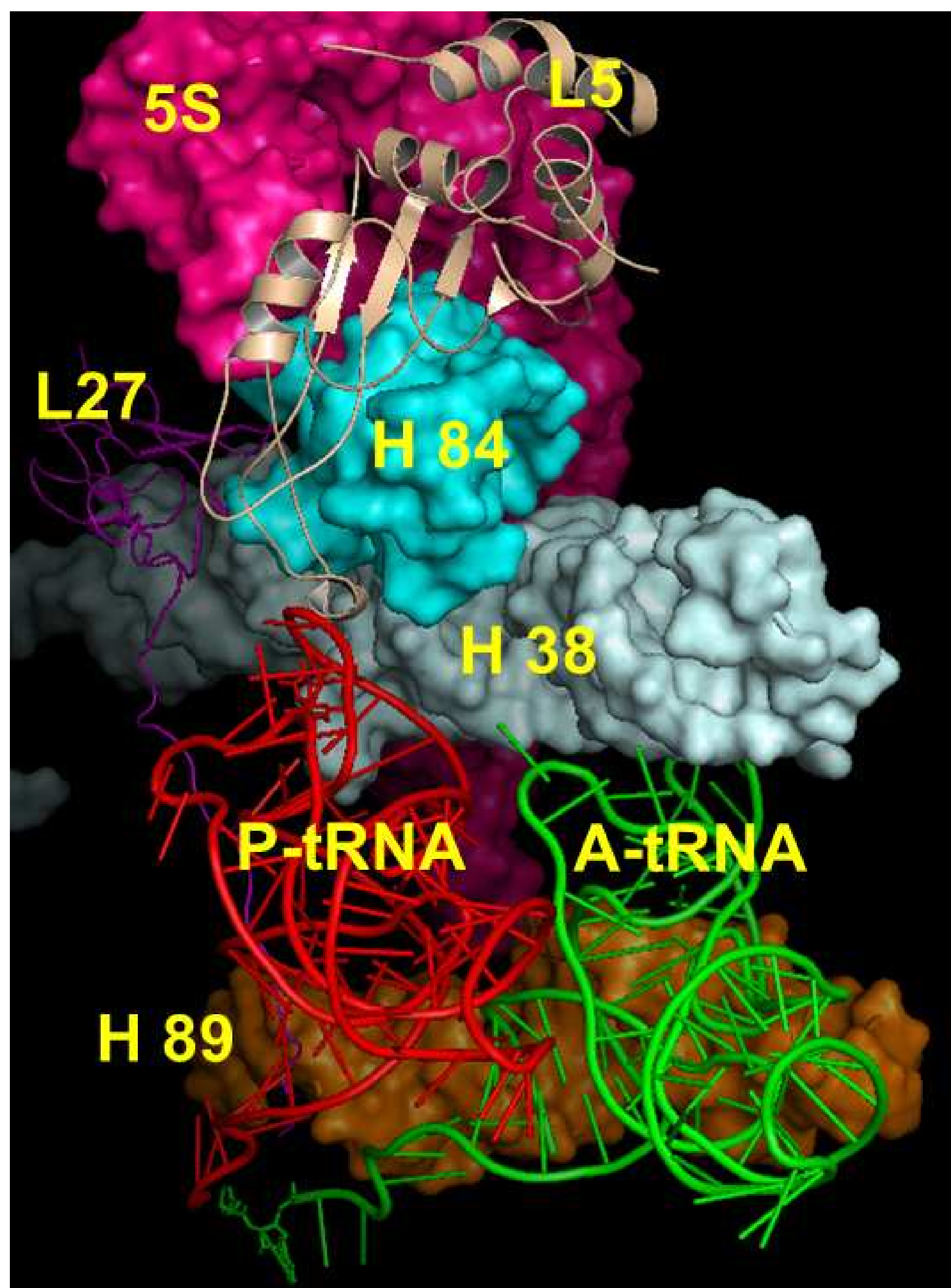

Figure S3. The possible network consists of L5/H84, tRNAs, L27, H38, H89 and 5sRNA, according to the X-ray coordinates in [1]. The swing of L5/H84 is observed for the formation of the P/E tRNA hybrid state [2]. This network implies the motion of L5/H84 to form the P/E state may cause L27 displacement and the A-site tRNA rotation, which yield the low FRET value for the A/A-P/E hybrid state.

Description of fitting with multiple Gaussian functions to deduce the FRET states compositions in single ribosomes.

For the time-lapse FRET traces in single ribosomes, the FRET state distributions can be fitted with three Gaussian functions (Figure 2 and Figure S4). The Gaussian function is determined by three parameters: A, the amplitude; x_0, the average position and σ^2, the variance. The absolute amount of each FRET state is calculated by the area underneath the Gaussian curve $(A*\sigma*(2\pi)^{1/2})$. The percentages are calculated by normalizing the sum of three FRET states into 1.

Statistically, the amplitudes varied freely because each trace contains varied data points and FRET composition. However, the Gaussian parameters x_0 and σ each follow a Gaussian distribution themselves. As shown in the lower panels of Figure S4, three Gaussians can be fitted for both the x_0 and σ. The left bottom plot of Figure S4 shows that the average values (deviation) for σ are: 0.01 (0.01), 0.04 (0.02) and 0.08 (0.04). The right bottom plot shows that the average values (deviation) for the fitted x_0 are: 0.25 (0.08), 0.46 (0.12) and 0.68 (0.1). These values agree well with the average positions fitted from the raw FRET values [3]. Together with the tight distribution of σ, these results indicate that Gaussian fittings on single FRET traces are reasonable. The average length of the traces is approximately 110 data points (the average bleaching time for the donor dye).

Figure S4.

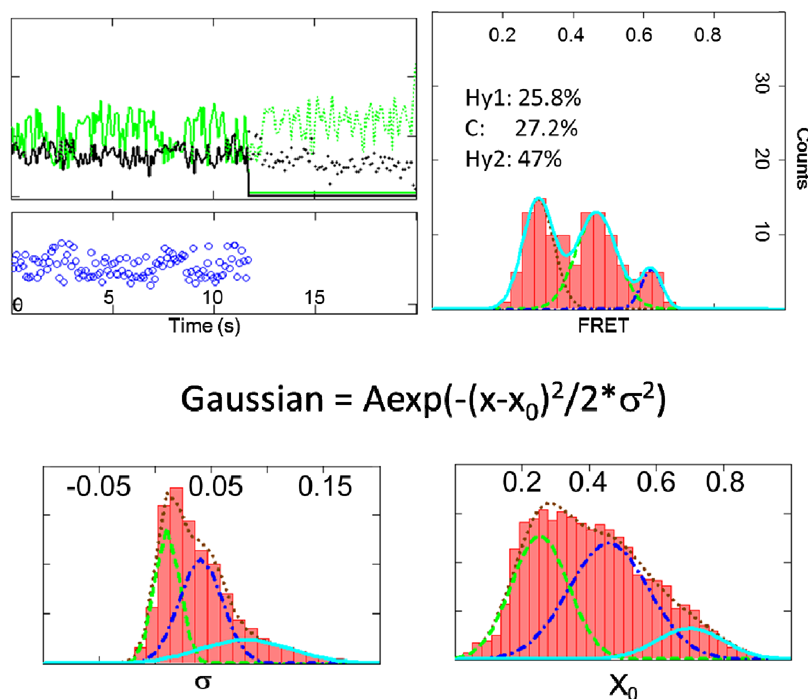

Figure S4. Top left: representative fluorescent intensity traces and the calculated FRET trace. Top right: Gaussian fittings of the FRET traces with approximately 110 points. The amount of the FRE states are calculated by the area beneath the Gaussian curves $(A*\sigma*(2\pi)^{1/2})$, and then normalized. Bottom left: Statistical distribution of the σ obtained from the Gaussian fittings. Bottom right: Statistical distribution of the X_0 obtained from the Gaussian fittings.

Figure S5.

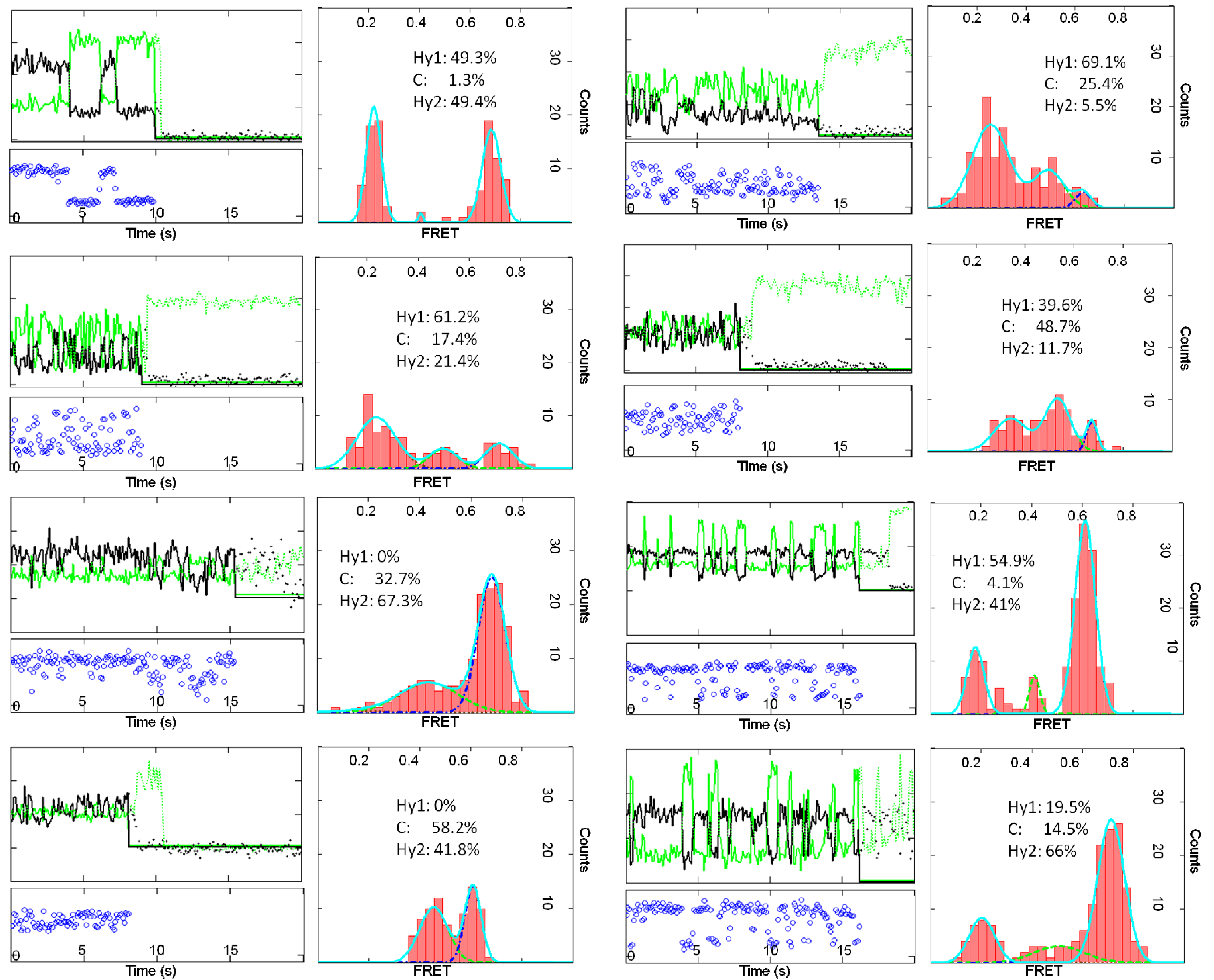

Figure S5. More examples of Wt-ribosome pre-translocation particles that exhibit different dynamics.

Figure S6.

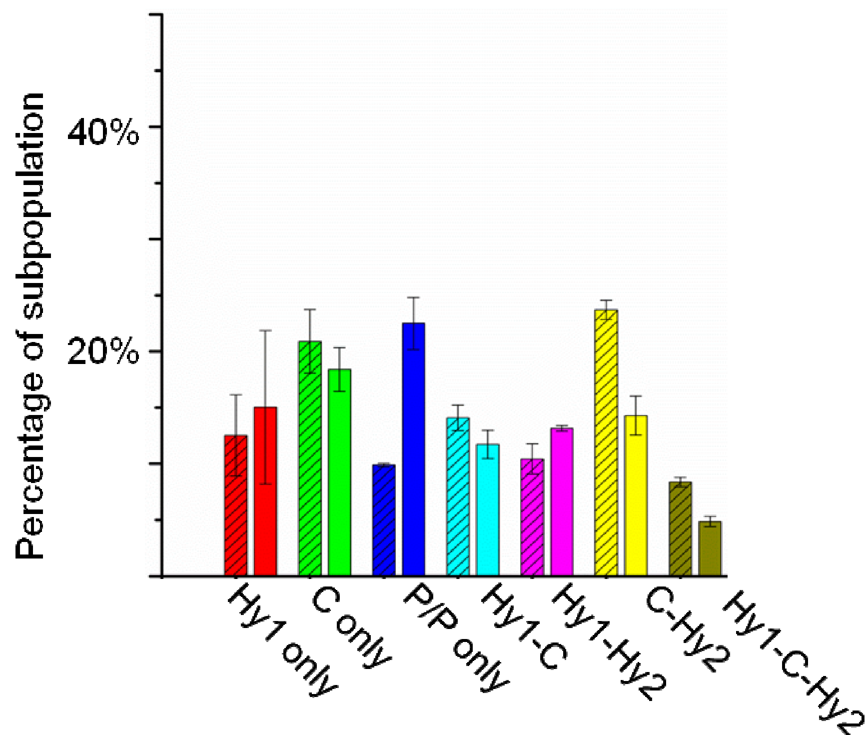

Figure S6. The subpopulation distributions for the Rb-K4E. The bars with the pattern are the data points for the pre-translocation complex; the bars with solid colors are the data points for the post-translocation complex. In contrast to Figure 3c, the formation of the P/P subpopulation is only approximately 20%, which is the lowest of all the ribosome species studied in this report.

1. Voorhees RM, Weixlbaumer A, Loakes D, Kelley AC, Ramakrishnan V (2009) Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. Nat Struct Mol Biol 16: 528-533.
2. Fischer N, Konevega AL, Wintermeyer W, Rodnina MV, Stark H (2010) Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy. Nature 466: 329-333.
3. Altuntop ME, Ly CT, Wang Y (2010) Single-molecule study of ribosome hierarchic dynamics at the peptidyl transferase center. Biophys J 99: 3002-3009.