Supporting Information
Shen et al. 10.1073/pnas.1002968107

Fig. S1. Stimulated GTPase reactions between SRP and wild-type or mutant FtsY, determined as described in Materials and Methods. The data were fit to the Michaelis–Menten equation, and gave $k_{cat}$ and $K_m$ values of 64.6 min$^{-1}$ and 0.940 μM for wild-type FtsY (○), 23.3 min$^{-1}$ and 27.7 μM for K399A (♦), 20.7 min$^{-1}$ and 1.87 μM for R402A (□), and 35.3 min$^{-1}$ and 2.37 μM for K406A (▲).

Fig. S2. Equilibrium titration of the SRP–FtsY–K399A complex formed in GppNHp using FRET. Nonlinear fits of data gave a $K_d$ value of 97.4 nM, consistent with the value of 106 nM calculated from the ratio of association and dissociation rate constants ($K_d = K_{off}/K_{on}$; Fig. 1 B and C). For comparison, the $K_d$ value for the wild-type SRP–FtsY complex was 92 nM from equilibrium titrations and 21–36 nM from the ratio of dissociation and association rate constants (ref. 18 and Fig. 1 B and C).
Fig. S3. Effect of FtsY mutations on the rate of complex assembly between mutant SRP(GAAU) and FtsY, determined from the $k_{\text{cat}}/K_m$ values in the stimulated GTPase assay as described in the text. The data were fit to Michaelis–Menten equation, and gave $k_{\text{cat}}/K_m$ values of $3.8 \times 10^5$, $4.1 \times 10^5$, $1.4 \times 10^5$, and $1.2 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$ for wild-type FtsY (○), FtsY-K399A (□), FtsY-E475K (●), and FtsY-T307W (▲), respectively.

Fig. S4. Dependence of Ffh–FtsY complex assembly kinetics on the NaCl concentration in the presence (A) and absence (B) of SRP RNA. Complex assembly rate constants were determined using the FRET assay as described in the text. All reactions were carried out in solutions that contain a constant concentration of 50 mM KOAc and 2 mM Mg(OAc)$_2$. Linear fits of data gave complex assembly rate constants of $7.37 \times 10^4$, $3.05 \times 10^4$ and $5.09 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ with 0, 100 and 200 mM NaCl, respectively, for reactions in the presence of the SRP RNA (A), and 159, 212 and 77 $\text{ M}^{-1} \text{ s}^{-1}$ with 0, 100 and 200 mM NaCl, respectively, for reactions in the absence of the SRP RNA (B).

Fig. S5. FtsY–Lys399 plays an essential role in cotranslational protein targeting. Upper SDS-PAGE analysis of the translocation of pPL by wild-type FtsY and mutant FtsY–K399A. pPL and PL denote the precursor and mature forms of the model substrate preprolactin, respectively. Lower Quantification of the results from SDS-PAGE.