# Supporting Information

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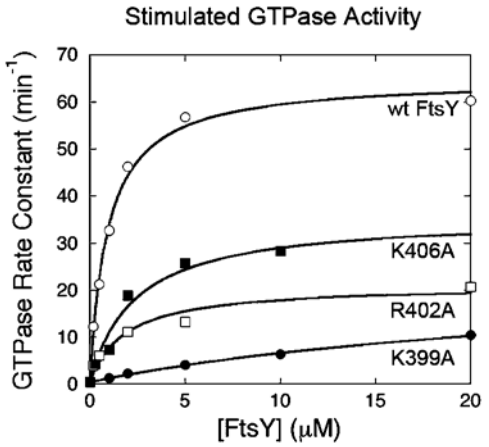


**Fig. S1.** Stimulated GTPase reactions between SRP and wild-type or mutant FtsY, determined as described in *Materials and Methods*. The data were fit to the Michaelis–Menten equation, and gave $k_{cat}$ and $K_m$ values of 64.6 $min^{-1}$ and 0.940 μM for wild-type FtsY (○), 23.3 $min^{-1}$ and 27.7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

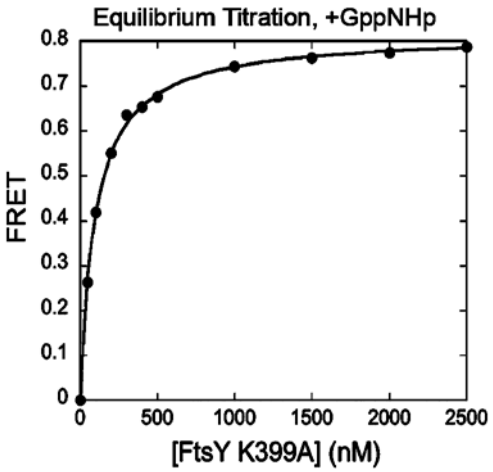


**Fig. S2.** Equilibrium titration of the SRP–FtsY–K399A complex formed in GppNHp using FRET. Nonlinear fits of data gave a $K_d$ value of 97.4 nM, consistent with the value of 106 nM calculated from the ratio of association and dissociation rate constants ($K_d = K_{off}/K_{on}$; Fig. 1 B and C). For 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).

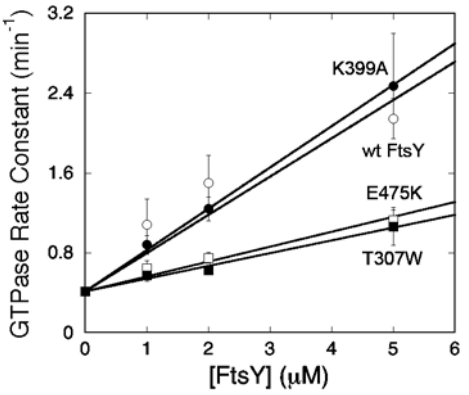


**Fig. S3.** Effect of FtsY mutations on the rate of complex assembly between mutant SRP(GAAU) and FtsY, determined from the $k_{cat}/K_m$ values in the stimulated GTPase assay as described in the text. The data were fit to Michaelis–Menten equation, and gave $k_{cat}/K_m$ values of $3.8 \times 10^5$, $4.1 \times 10^5$, $1.4 \times 10^5$, and $1.2 \times 10^5$ $M^{-1}$ $min^{-1}$ for wild-type FtsY (○), FtsY-K399A (●), FtsY-E475K (□), and FtsY-T307W (■), respectively.

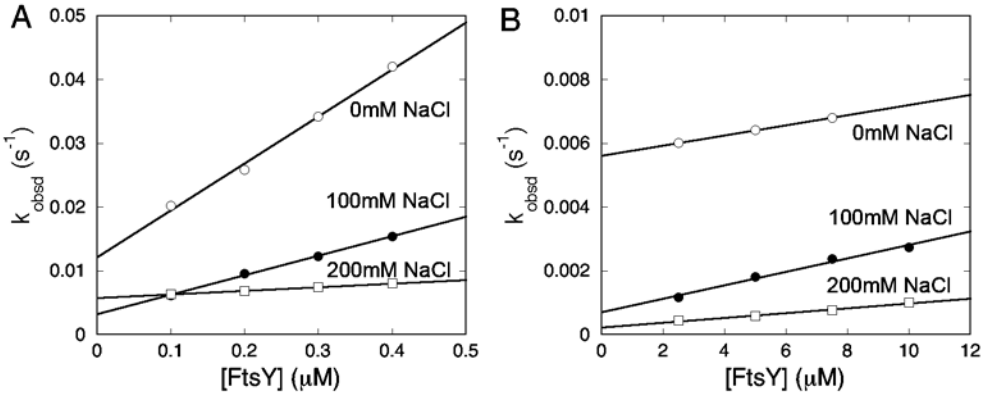


**Fig. S4.** Dependence of Ffh–FtsY complex assembly kinetics on the NaCl concentration in the presence (*A*) and absence (*B*) of SRP RNA. Complex assembly rate constants were determined using the FRET assay as described in the text. All reactions were carried out in solutions that contain a constant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

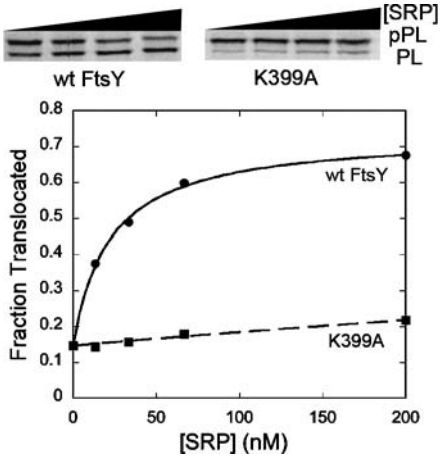


**Fig. S5.** FtsY–Lys399 plays an essential role in cotranslational protein targeting. *Upper* SDS-PAGE analysis of the translocation of pPL by wild-type FtsY and mutant FtsY–K399A. pPL and PL denote the precursor and mature forms of the model substrate preprolactin, respectively. *Lower* Quantification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