Supplemental material

JCB

Kuhn et al., http://www.jcb.org/cgi/content/full/jcb.201502103/DC1

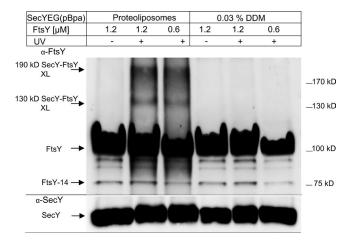


Figure S1. **The SecY-FtsY interaction requires the presence of lipids.** SecYEG(pBpa) PLs or SecYEG(pBpa) in detergent solution (0.03% DDM) were incubated with different FtsY concentrations and UV activated. Samples after UV activation were directly separated by SDS-PAGE without prior carbonate extraction. After Western blotting, the membrane was decorated with antibodies against FtsY (top). As a loading control, the lower part of the gel was decorated with antibodies against SecY (bottom). Conditions for cross-linking were as described in the legends for Figs. 1 and 2.

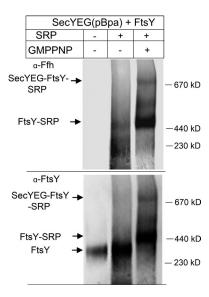


Figure S2. **Detection of FtsY-SRP and SecYEG-FtsY-SRP complexes by blue native PAGE.** SecYEG(pBpa) PLs (final SecYEG concentration 10 nM) were incubated with FtsY (1.2 μ M) in the absence or in the presence of SRP (1 μ M) and GMP-PNP (0.5 mM). After UV exposure, the samples were solubilized, Blue native–PAGE loading dye was added to a final concentration of 10% (vol/vol), and the samples were loaded on a 4%–16% native PAGE. After Western transfer, the membrane was decorated with antibodies against Ffh (top) and FtsY (bottom). Indicated are FtsY, the FtsY–SRP complex at ~400 kD, and the SecYEG–FtsY–SRP complex at ~700 kD (Braig et al., 2011). Note that migration on blue native–PAGE does not correspond to the predicted mass of a complex because it is influenced by the 3D structure of the complex and the amounts of detergent and Coomassie blue bound to it (Heuberger et al., 2002).

References

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Heuberger, E.H., L.M. Veenhoff, R.H. Duurkens, R.H. Friesen, and B. Poolman. 2002. Oligomeric state of membrane transport proteins analyzed with blue native electrophoresis and analytical ultracentrifugation. *J. Mol. Biol.* 317:591–600. http://dx.doi.org/10.1006/jmbi.2002.5416