Supporting Information

Dalal et al. 10.1073/pnas.1117783109

SI Materials and Methods

Cloning of pBAD22-HisEY$_{FF}$-YG and pBAD22-HisEY$_{FF}$-Y$_E$G. To express two different SecY complexes from the same plasmid, a second *secY* gene together with an identical ribosome binding site were introduced into plasmid pBAD22-HisEYG, in-between *secY* and *secG*. Briefly, the restriction site *Sac*I and *Xba*I were inserted by site-directed mutagenesis at the 3′ end of *secY* and 5′ end of *secG*, respectively. Next, a second *secY* flanked by a ribosome binding sequence was generated by PCR amplification and inserted in-between *SacI* and *XbaI*. The resulting plasmids pBAD22-HisEY$_{FF}$-YG and pBAD22-HisEY$_{FF}$-Y$_E$G were verified by sequencing analysis and restriction mapping.

Reconstitution of SecY or SecY-Fused Dimers into Nanodiscs. Reconstitution of the SecY complex into MSP1 was performed as previously described (1, 2) at a molecular ratio SecYEG : MSP1 : lipids of 1 : 5 : 40. Reconstitutions of the SecYEG dimer into MSP3 were performed at a molecular ratio of 1 : 3 : 20. The covalently linked SecYEG dimer (SecYY; 3) was reconstituted into MSP3 or MSP2N2 at a molecular ratio of 1 : 5 : 40. Nanodiscs containing only phosphatidylglycerol (PG) or *Escherichia coli* lipids were prepared at a molecular ratio of MSP3 : lipids of 1 : 20. Purification of the reconstituted discs was by gel-filtration chromatography on a Superdex 200 HR10/30 column equilibrated in 50 mM Tris·HCl pH 7.9, 100 mM NaCl, 5% glycerol (TSG buffer).

Sucrose Density Purification of Nanodiscs. Sucrose density purification of SecY fusion complexes in MSP2N2 was performed by layering the nanodisc preparation on top of a 10 mL sucrose gradient (6–13% in TSG buffer), followed by centrifugation at 188,000 × *g* in a Beckmann SW41 rotor (17 h, 4 °C). Fractions (0.5 mL) were collected top-down and analyzed by native-PAGE (see Fig. S7).

Labeling of PhoA1-202 with Fluorescent Dye. Dye-labeling of PhoA1-202 (100 μg) was performed by incubation with Alexa Fluor 680 (40 ng/μL; Invitrogen Molecular Probes) in 50 mM Tris·HCl pH 7.9, 6 M urea, 1 mM EDTA, and 5 μM Tris(2-carboxyethyl) phosphine buffer for 2 h at room temperature. The reaction was quenched with 1 mM DTT, and excess dye was removed by gel-filtration chromatography in 50 mM Tris·HCl pH 7.9, 5 M urea, and 1 mM DTT.

1. Alami M, Dalal K, Lelj-Garolla B, Sligar SG, Duong F (2007) Nanodiscs unravel the interaction between the SecYEG channel and its cytosolic partner SecA. *EMBO J* 26:1995–2004.
2. Dalal K, Duong F (2010) Reconstitution of the SecY translocon in nanodiscs. *Methods Mol Biol* 619:145–156.
3. Duong F (2003) Binding, activation and dissociation of the dimeric SecA ATPase at the dimeric SecYEG translocase. *EMBO J* 22:4375–4384.

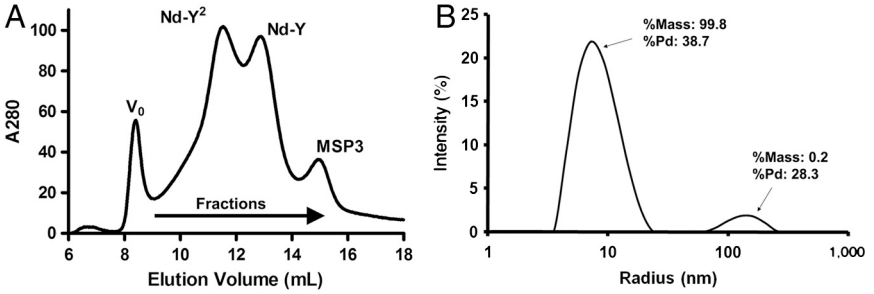

Fig. S1. Example of gel-filtration analysis of the SecY complex reconstituted in nanodiscs. (*A*) The SecY complex reconstituted with MSP3 [~500 μg, dioleoylphosphatidylglycerol (DOPG) lipids] was separated by size-exclusion chromatography on a Superdex 200 HR10/30. (*B*) Dynamic light scattering analysis of a purified SecY dimer in nanodiscs (i.e., fraction 7 shown in Fig. 2*B*). The mass fraction (%Mass) and polydispersity (%Pd) is indicated above each peak.

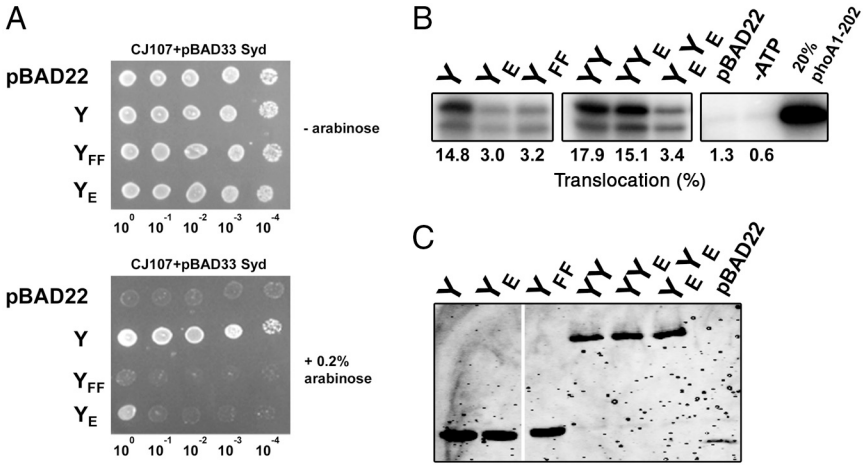

Fig. S2. In vivo and in vitro activity of the SecY mutant channels employed in this study. (*A*) *E. coli* CJ107 (*secY24*) transformed with the indicated plasmids was grown for 6 h in LB broth, then serial diluted and spotted on LB-agar plates containing 0.2% arabinose to induce plasmid expression of Syd and the indicated SecY complex. Overproduction of Syd is lethal in CJ107 because Syd destabilizes the already thermo-sensitive SecY24 complex, which becomes rapidly proteolysed (1, 2). In this way, the complementation ability of the plasmid encoded SecY complexes could be tested at 37 °C. (*B*) The in vitro protein translocation activity was assayed using approximately 25,000 c.p.m. of ^{125}I-labeled PhoA1-202 as described in *Materials and Methods* in the main text. The lane pBAD22 refers to inner membrane vesicles (IMVs) prepared from bacteria expressing endogenous level of SecYEG. Right lane shows 20% of the input material. The percent of translocated PhoA1-202 was determined by densitometry using the ImageJ software. (*C*) Western blotting analysis of IMVs using a polyclonal antibody directed against SecY.

1. Dalal K, et al (2009) Structure, binding, and activity of syd, a SecY-interacting protein. *J Biol Chem* 284:7897–7902.
2. Shimoike T, et al (1995) Product of a new gene, syd, functionally interacts with SecY when overproduced in *Escherichia coli*. *J Biol Chem* 270:5519–5526.

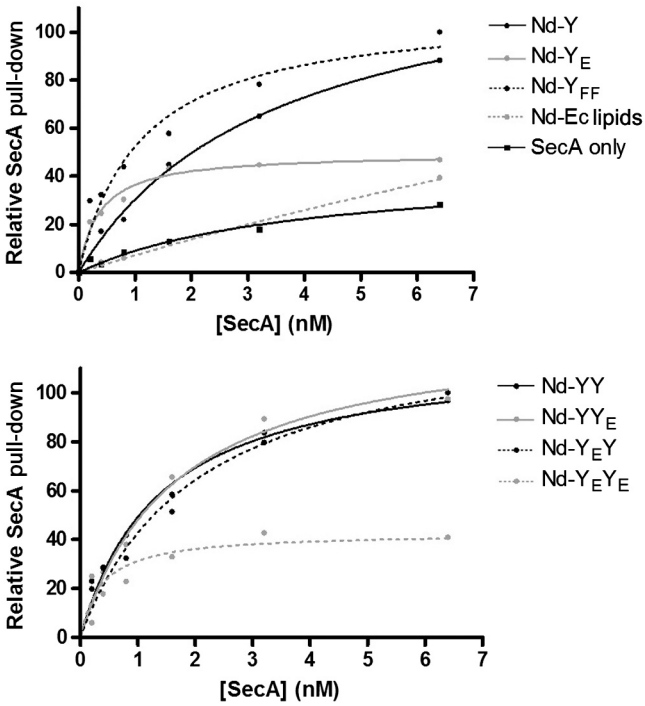

Fig. S3. Pull-down of ^{125}I-labeled SecA with nanodiscs. The amount of bound SecA from affinity pull-down experiments in Fig. 3*C* was quantified by densitometry using ImageJ software. One hundred percent is defined as the density observed after pull-down of 6.4 nM ^{125}I-SecA using Nd-Y_EY.

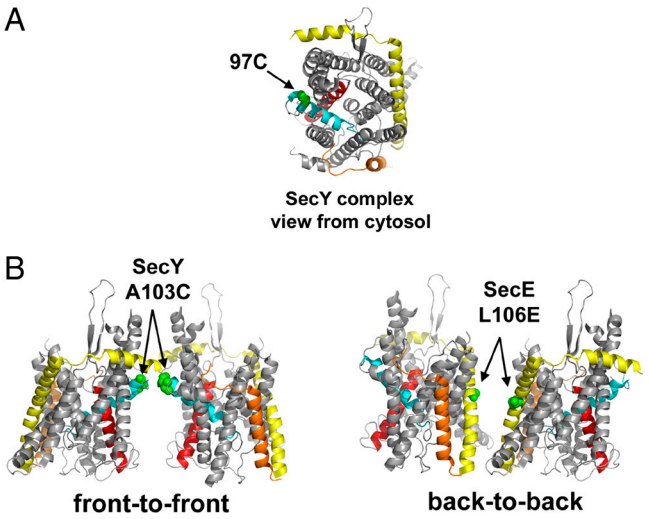

Fig. S4. Position of cysteine residues introduced into the SecY complex. (*A*) Top view (from the cytosol) of the SecY complex with position 97 indicated on TMS2 in the signal sequence binding site. (*B*) Hypothetical front-to-front and back-to-back representations of the SecY dimer (side view). The SecY complex is colored as follows: gray, SecY; yellow, SecE; orange, SecG; cyan, TMS2; red, TMS7; green, cysteine residue. The representations are based on SecYEβ structure from *Methanococcus jannaschii* [Protein Data Bank (PDB) ID code 1RHZ].

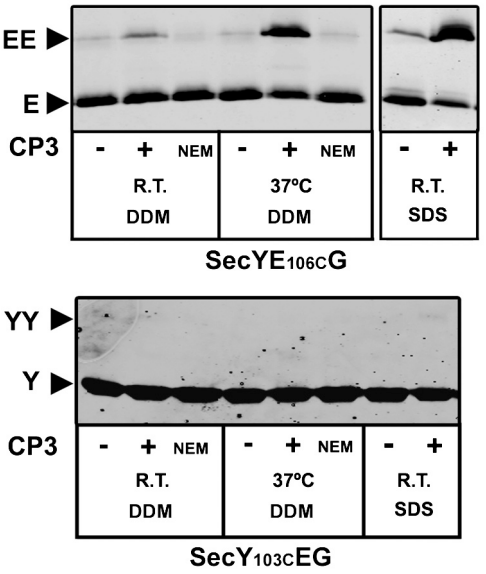

Fig. S5. Cross-linking of SecE-L106C and SecY-A103C in detergent solution. The purified SecY complex in detergent solution (2 μg in 0.03% dodecyl maltoside) was incubated for 5 min at the indicated temperature or in the presence of 0.2% SDS followed by incubation with 0.2 mM CP^3 (2 min, room temperature) and quenching with *N*-ethylmaleimide (NEM) (10 mM). Immuno-dectection using anti-SecE or anti-SecY antibodies were employed to increase the detection limit of the protein cross-links.

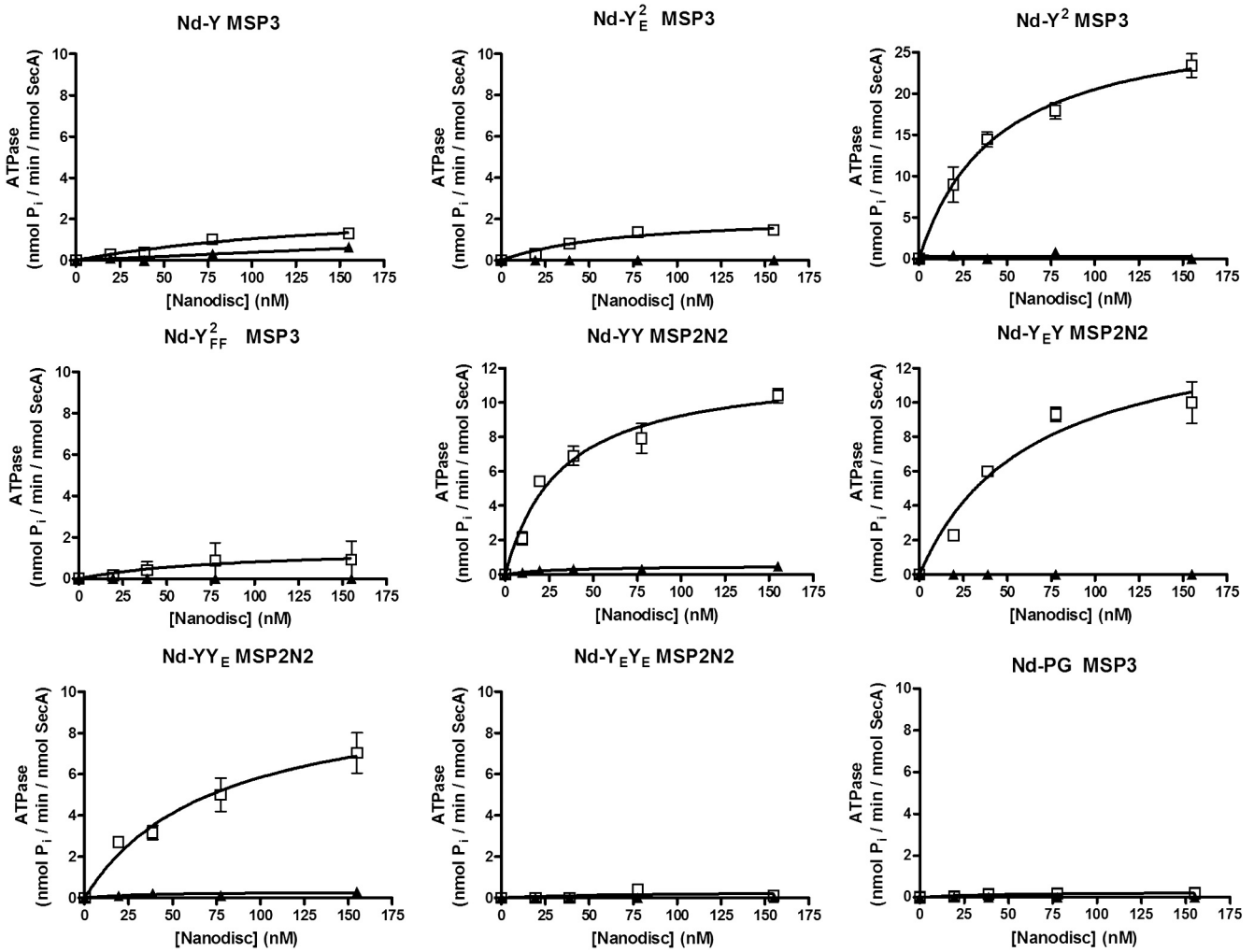

Fig. S6. SecA translocation ATPase enzyme kinetics. Initial rates of SecA translocation ATPase were determined in the presence of the indicated concentrations of purified nanodiscs, using the malachite green colorimetric assay. Reactions were performed with 0.2 μM SecA, 0.8 μM PhoA1-202 (square) or L14R signal sequence mutant (black triangle) over a range 30 min at 37 °C. The results were fitted to a one site quadratic binding equation to determine k_{cat} values (in Fig. 2 *C* and *D*; Fig. 3*D*) as described in *Materials and Methods* in the main text. Initial rates were determined at least three times, which reflect the error bars shown at each point.

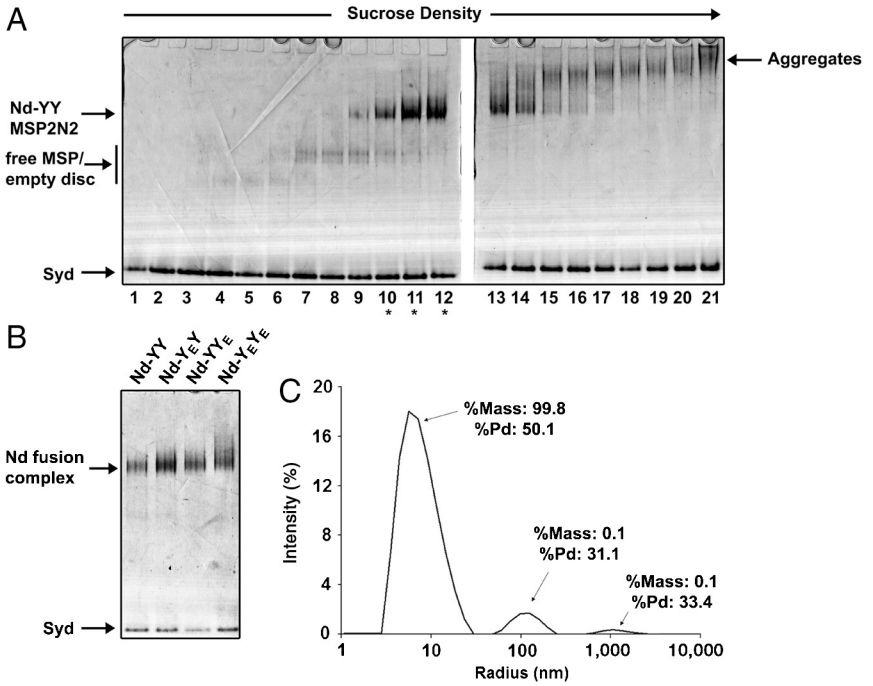

Fig. S7. Purification of the Nanodisc-SecY dimer by sucrose gradient centrifugation. (*A*) The covalently linked SecY dimer reconstituted with the MSP2N2 scaffold protein and PG lipids was loaded on top of a 6–13% sucrose gradient. After centrifugation (188,000 × *g*, 17 h, 4 °C), equal fractions were collected and analyzed by native-PAGE in the presence of Syd. Translocation ATPase measurements in Fig. 3*D* were performed on the fractions labeled with an asterisk. The fused SecY dimer supported the highest translocation ATPase when reconstituted with the MSP2N2 scaffold protein. (*B*) Nanodiscs bearing the indicated SecY mutants were purified as in *A*. (*C*) Assessment of the purity of the Nd-YY complex by dynamic light scattering. The trace shows that the nanodisc complex (~8 nm radius) is 99.8% pure from any liposomes (50–100 nm) or large aggregates (>100 nm).

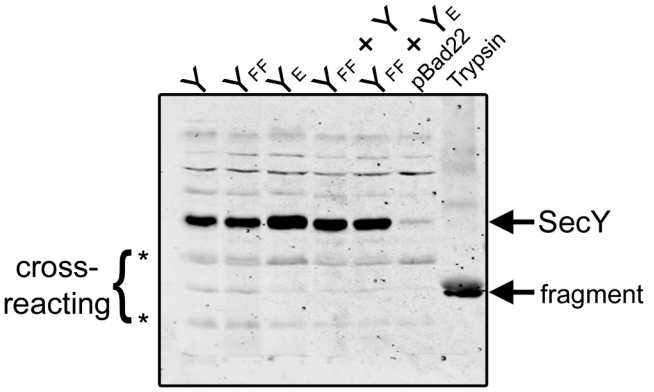

Fig. S8. Analysis of the IMVs (~3 μg) employed in the translocation experiments described in Fig. 6 *C* and *D*. Immuno-detection was using a polyclonal antibody directed against SecY. The large cytoplasmic loop of SecY is sensitive to proteolysis. On the right lane, the detergent purified SecYEG complex (1 μg) was incubated with trypsin (~300 ng for 15 min at 4 °C), followed by precipitation with 17% trichloroacetic acid prior to loading on the gel. This band is absent from IMVs. The bands labeled with an asterisk are cross-reacting proteins with the SecY antibody.