Whitehouse et al., http://www.jcb.org/cgi/content/full/jcb.201205191/DC1

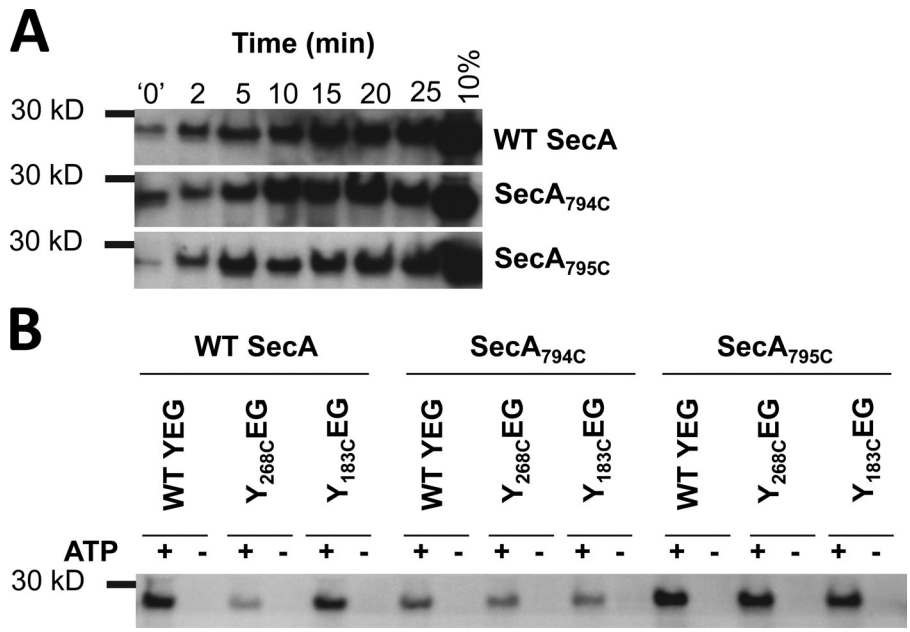

Figure S1. **Representative blots and time course of protein translocation activity through SecYEG by wild-type and mutant SecA.** (A) Relative levels of preprotein translocation (compared with 10% of the total input) of 0.3 µM wild-type and mutant SecA in the presence of 0.3 µM wild-type SecYEG proteoliposomes, with 0.7 µM proOmpA over 25 min at 25°C. t = 0 represents translocation after initial mixing of reaction components, ~10 s. See Fig. 2 and Materials and methods for further details. (B) Representative Western blot of comparative translocation assay of uncross-linked SecA and SecYEG mutants and wild types (quantified in Fig. 2 B, bottom). Calculated molecular weight of proOmpA Δ176-296 = 25.4 kD.

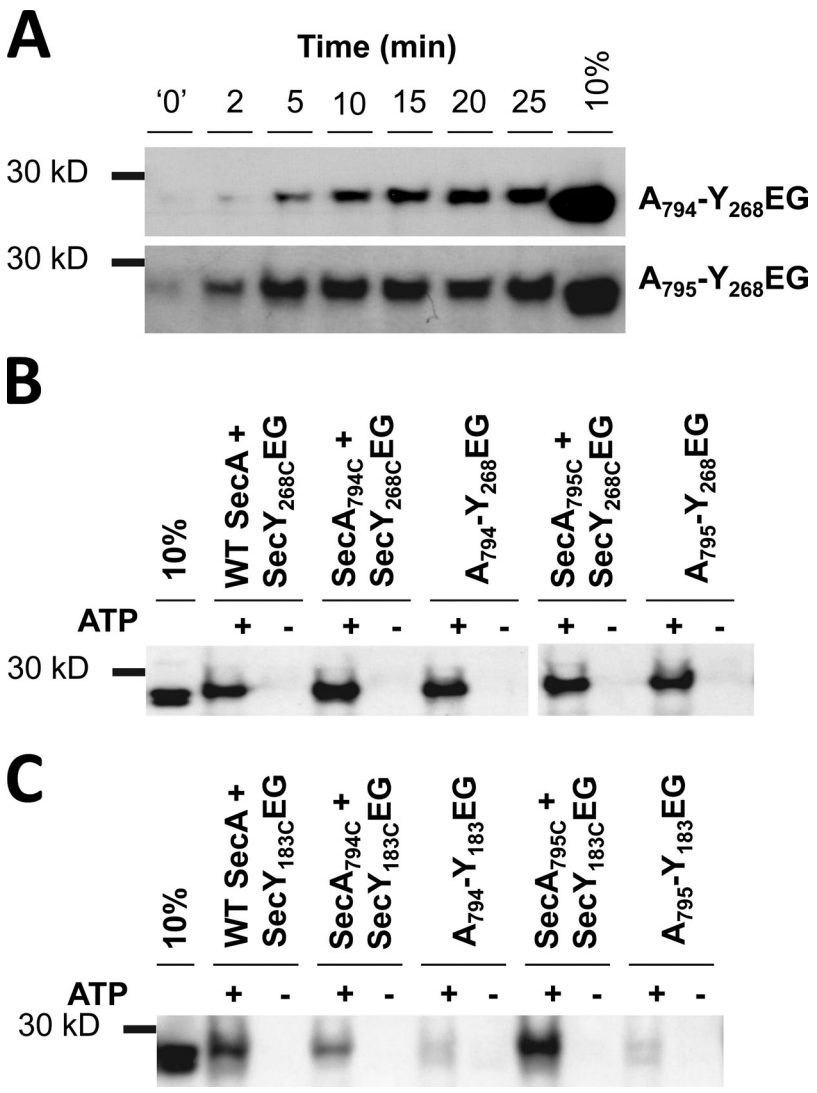

Figure S2. **Representative blots and time course of protein translocation activity through the cross-linked SecA-YEG complex.** (A) Relative translocation activities of 0.3 µM $SecA_{794/795}$–$SecY_{268}EG$ cross-linked complexes reconstituted into phospholipid vesicles, with 0.7 µM proOmpA over 25 min at 25°C. t = 0 represents translocation after initial mixing of reaction components, ~10 s. See Fig. 5 and Materials and methods for further details. (B and C) Representative Western blot of comparative translocation assay of various SecA and SecYEG wild-type, mutant, and cross-linked complexes (quantified respectively in Fig. 5, A and B, bottom). Calculated molecular weight of proOmpA Δ176-296 = 25.4 kD.

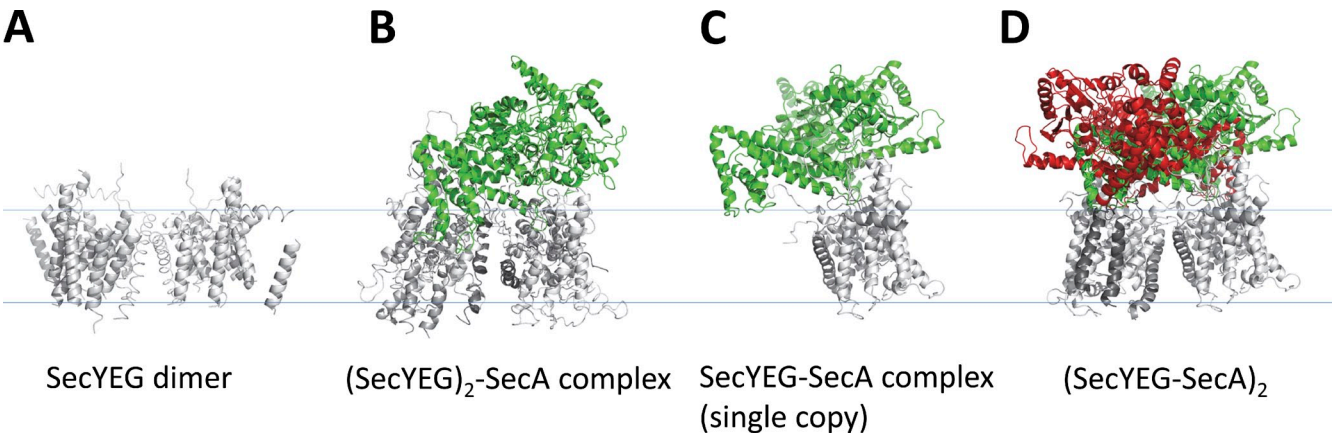

Figure S3. **Dimeric arrangement of SecYEG.** Structures of various arrangements of SecYEG and SecA in ribbon representation, shown from the side of the membrane (blue lines) with the cytosolic regions uppermost: SecYEG (gray-white), SecA (A–D, green; D, red). (A) *E. coli* SecYEG dimer model according to the membrane-bound structure determined by 2D-crystallography (Breyton et al., 2002). (B) Model of the *E. coli* SecYEG dimer bound to SecA (Deville et al., 2011), based on the structures of the known arrangement of the active SecYEG dimer (Breyton et al., 2002; Deville et al., 2011) and the structure of the SecA–YEG complex (Zimmer et al., 2008). (C) x-ray structure of the *T. maritima* SecA–YEG complex (Zimmer et al., 2008). (D) Hypothetical combination of two cross-linked SecA–YEG complexes about the known dimeric interface of SecYEG. The different SecAs are shown in green and red to illustrate the extensive clashes that make this arrangement highly implausible.

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