SUPPORTING INFORMATION

The SecA ATPase motor protein binds to *Escherichia coli* liposomes only as monomers

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Supplementary Figures

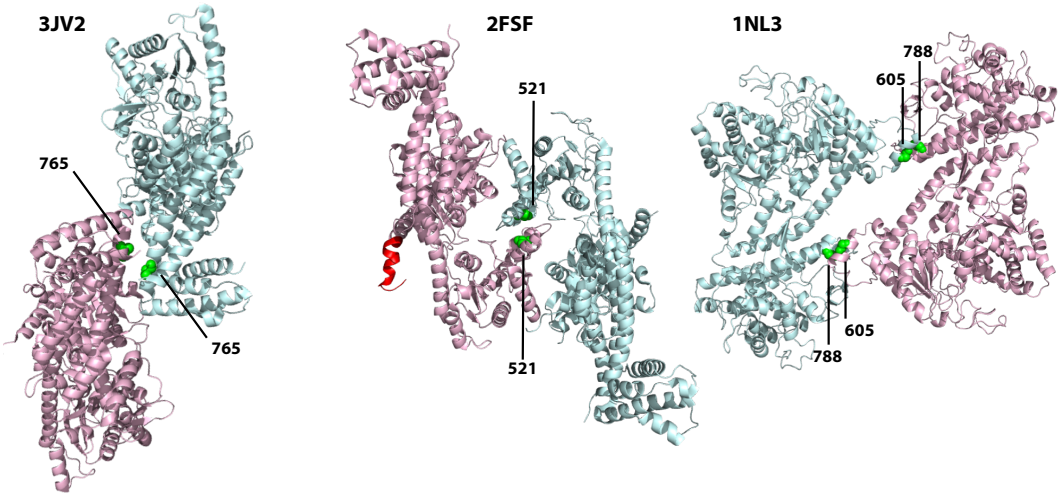

Figure S1. Construction of cysteine mutants. Cysteines were engineered into a cys-free SecA construct to stabilize the different dimers under oxidizing conditions. The stabilizing pairs of cysteines for each dimer are highlighted in green.

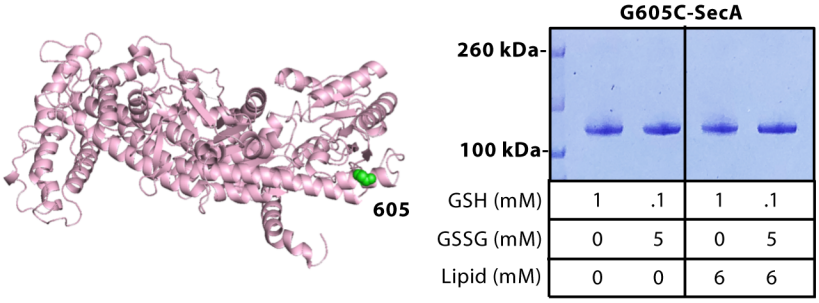

Figure S2. The 1NL3 dimer interface requires two cysteine residues, Cys788 and Cys605, to stabilize the dimer with two disulfide bridges between the protomers (Figure S1). The introduction of only one cysteine residue, Cys605, did not result in the formation of a dimer, even in the presence of the GSSG oxidizer, demonstrating the specificity of our protocol. GSH: reduced glutathione. GSSG: oxidized glutathione. Lipid: LUV formed from *E. coli* lipids.

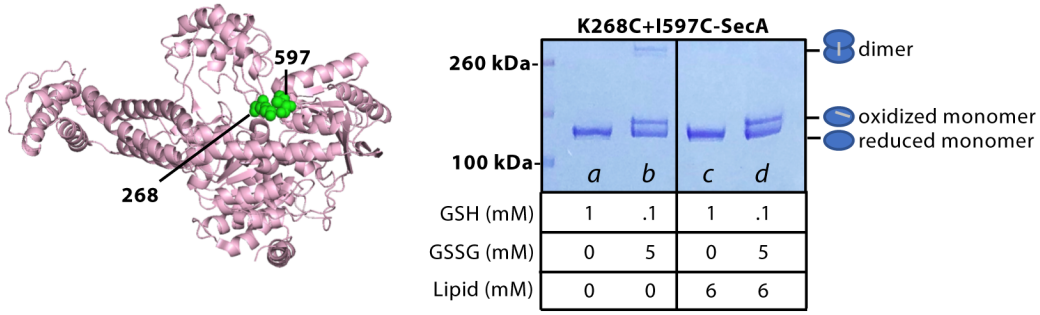

Figure S3. To address the question of whether or not LUV might interfere with disulfide bridge formation, we used a SecA construct with a pair of cysteines within SecA that seemed unlikely to form strong disulfide bridges between monomers. We used a construct designed by Chatzi et al. [1] that stabilized SecA in the so-called 'locked closed' (LC) conformation. The intra-monomer disulfide bridge in the LC construct is between Cys268 & Cys597. We chose this construct because it migrates on gels at an apparent molecular weight that is higher than the wild-type protein (see Figure S4 of Chatzi et al.). Thus, with a short period of disulfide oxidation, we expected to see two bands on the gel corresponding to the two forms of monomer. Lane *a* shows the migration of SecA in the absence of oxidizer (GSSG) and LUV. In the absence of lipid and the presence of GSSG (introduced by exchanging the buffer using a desalting column, see Materials and Methods section 2.6), lane *b* reveals two strong monomeric bands, one of them being the oxidized monomer, and a very weak dimer band. In the presence of LUV and the absence of the oxidizer, lane *c* reveals a single monomeric band. In the presence of lipid and GSSG, lane *d* reveals two bands exactly as in lane *b*. This demonstrates that the LUVs do not interfere with intra-monomeric disulfide bridge formation. Interestingly, not a trace of dimeric SecA is seen in lane *d*, consistent with the disruption of SecA dimers by LUV.

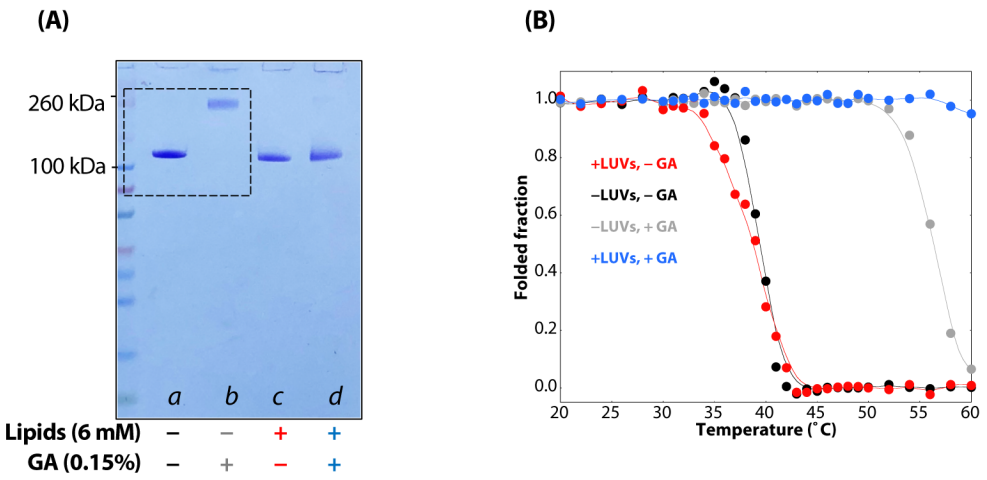

Figure S4. Non-specific cross-linking of WT-SecA using glutaraldehyde (GA) under various conditions. **A**. Coomassie-blue stained SDS-PAGE of SecA protein (1 μM) in solution in the absence (−) or presence (+) of 0.15% GA (15 seconds exposure, before halting the reaction with 100 mM tris-HCl pH 7.0) and in the absence (−) or presence (+) of lipid vesicles. The section of the gel used in Figure 2 of the main text is indicated by the dashed lines. In the absence of LUV and GA (lane *a*), only monomeric SecA is seen, while in the presence of GA the monomers are cross-linked to form dimers (lane *b*). Only monomers are seen in the presence of LUV with or without GA (lanes *c* and *d*). **B.** GA-crosslinking of SecA can occur on the surface of lipid vesicles as revealed by an increase in thermal stability. Thermal unfolding of SecA in the presence and absence of vesicles and GA was measured by monitoring SecA tryptophan fluorescence. Folded fractions were determined by assuming that SecA (1 μM) was fully folded at 20˚C and fully unfolded at 80˚C. The tryptophan fluorescence at 340 nm was then set to 1 and 0, respectively, after correction for the temperature dependence of the fluorescence intensity. In solution (no LUVs and no GA), SecA is about 50% unfolded at ~39°C (black curve), as previously observed [2, 3]. After partitioning into LUVs (POPC:POPG:CL=0.7:0.2:0.1) for 30 minutes, the thermal transition temperature is little changed (red curve). After crosslinking SecA in solution (0.15%, 15 seconds before halting the reaction with 100 mM Tris-HCl pH 7.0), the thermal stability of SecA is significantly increased, probably because of internal non-specific crosslinking (grey curve). After partitioning (30 minutes), the GA-treatment also increases SecA thermal stability (blue curve), demonstrating that GA-crosslinking also occurs on the surface of LUVs.

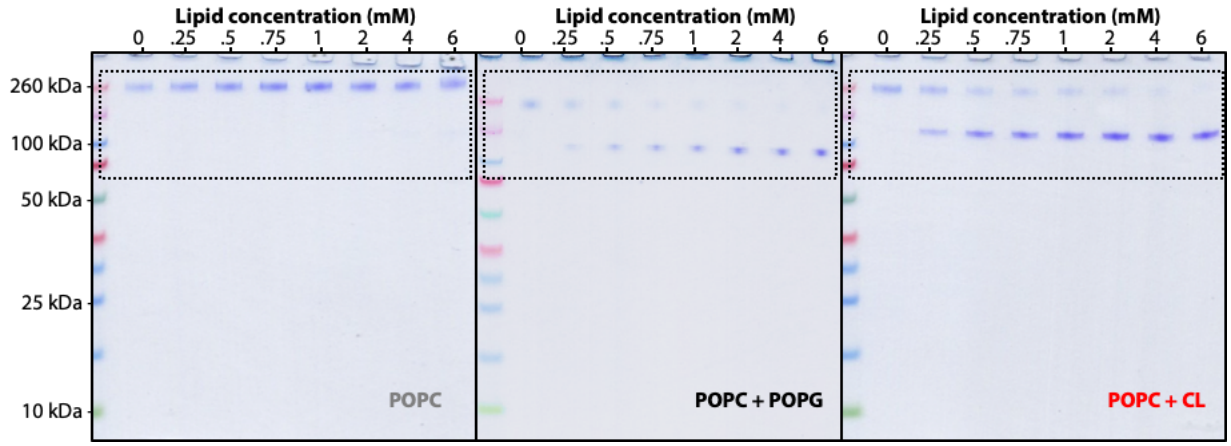

Figure S5. Dimeric SecA dissociates into monomers upon binding to LUVs. Following binding to LUV (30 minutes at 37˚C, composition as noted), SecA (1 μM) was cross-linked by GA (0.15%, 15 seconds, before halting the reaction by the addition of an excess of 100 mM Tris-HCl pH 7.0) and the resulting samples were loaded onto a denaturing gel. The dashed regions are used for Figure 3B.

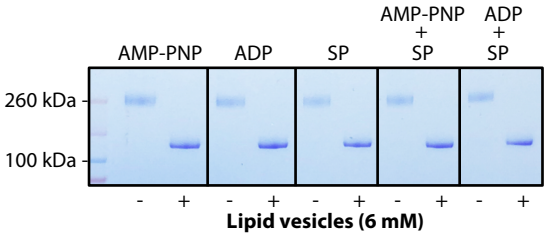

Figure S6. Effect of nucleotides and a pre-protein signal peptide on the oligomeric state of SecA in the presence and absence of vesicles. SDS-PAGE of WT-SecA (1 μM) incubated with AMP-PNP (5 mM), ADP (5 mM), and/or the signal peptide (SP) of prePhoA (5 μM) in the presence or absence of 6 mM LUVs made from POPC:POPG (0.7:0.3). After 30 minutes at 37˚C, cross-linking by GA (0.15 %, 15 seconds before the reaction was stopped by the addition of an excess of 100 mM Tris-HCl pH 7.0).

References

[1] K.E. Chatzi, M.F. Sardis, A. Tsirigotaki, M. Koulaki, N. Šoštarić, A. Konijnenberg, F. Sobott, C.G. Kalodimas, S. Karamanou, A. Economou, Preprotein mature domains contain translocase targeting signals that are essential for secretion, J. Cell Biol., 216 (2017) 1357-1369.

[2] G. Roussel, E. Lindner, S.H. White, Stabilization of SecA ATPase by the Primary cytoplasmic salt of *Escherichia coli*, Protein Sci., 28 (2019) 984-989.

[3] G. Roussel, S.H. White, Binding of SecA ATPase monomers and dimers to lipid vesicles, Biochim. Biophys. Acta, 1862 (2020) 183112.