SUPPLEMENTARY INFORMATION

TOPOLOGY OF THE SecA ATPase BOUND TO LARGE UNILAMELLAR VESICLES

Guillaume Roussel[†], Eric Lindner, and Stephen H. White*

Department of Physiology and Biophysics, University of California, Irvine, Irvine, California 92697-4560

*To whom correspondence should be addressed: stephen.white@uci.edu

[†]Present address: Laboratory of Molecular Bacteriology, Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven, University of Leuven, Leuven, Belgium.

Cysteine Position	Source
G11C	[49-51]
S12C	[49]
T17C	[49]
A30C	[51]
S39C	This work
T47C	[50]
G160C	This work
S264C	[51]
S300C	[51]
T340C	[51]
S350C	[51]
T470C	[52]
A521C	[53]
T530C	[51]
S600C	[52]
G605C	[53]
S636C	[54]
S661C	[51]
S670C	[55]
S696C	[52]
A721C	This work
G788C	[52]
A795C	[56]
S827C	[52]
S890C	This work
S896C	[51]

Table S1. SecA constructs used in this study. Except for those noted as “this work”, we used Cys constructs reported earlier by other labs.

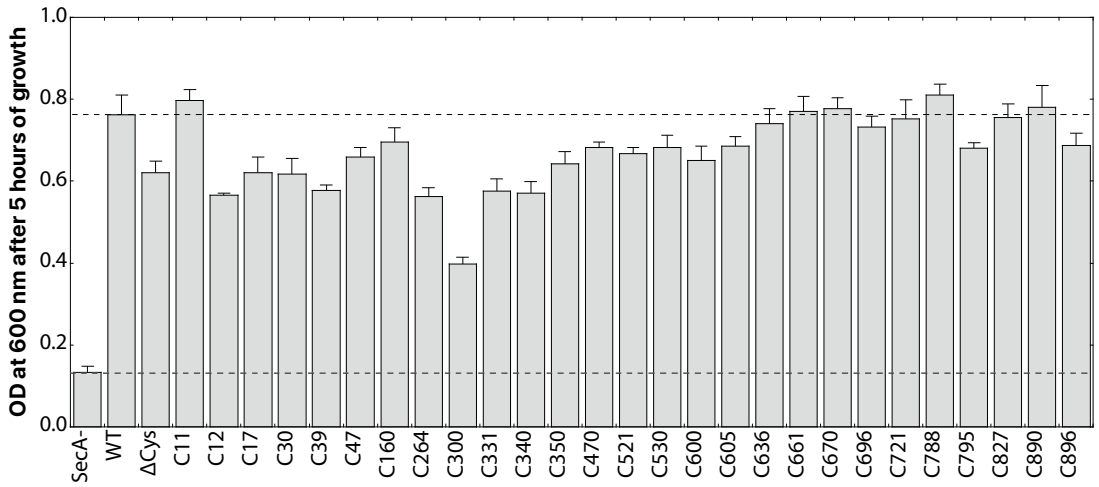

Figure S1 – **Complementation assay of mono-cysteine SecA-mutants.** *E. coli* strain EO527, a gift from Ross Dalbey, was used for the assay. In this strain SecA is under the control of the arabinose promoter/operator and was incorporated into the chromosome. Plasmids (pET-21) containing the various mutations were transformed in EO527 and were grown overnight (37°C) in LB media in the presence of 0.4% arabinose. Aliquots (500 µl) from each mutant were centrifuged and pellets were washed twice with 500 µl PBS buffer to remove the arabinose from the media (depletion condition). A 1:1000 dilution was used in fresh LB media without arabinose using 24 well plates. The optical densities (OD_{600}) were determined for each mutant in quadruplicate (4 wells for each mutant (standard deviation) after 5h of growth (37° C). The negative control contains an empty pET-21 plasmid ($SecA^-$). It shows that the depletion strain EO527 is not able to grow in the absence of arabinose (absence of SecA). The positive control contains a pET-21 plasmid with SecA-WT (WT) with a C-terminal His6-tag. This construct can complement strain EO527 (growth in the absence of arabinose).

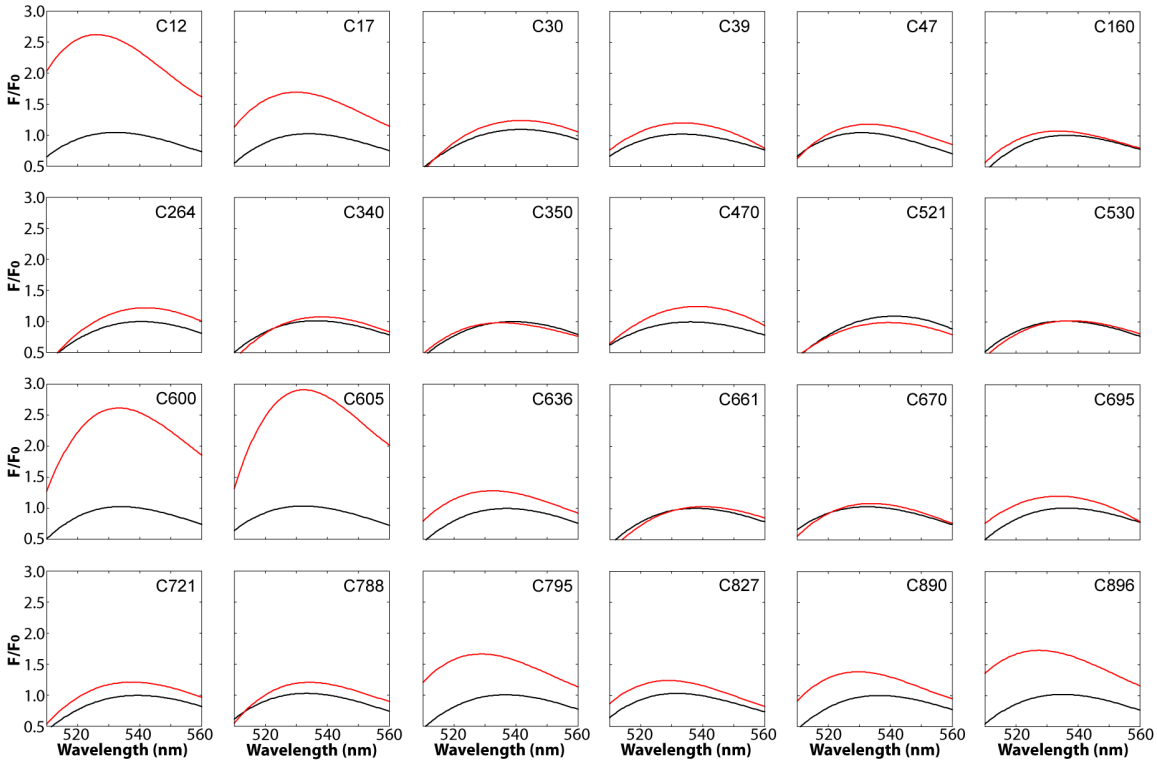

Figure S2 – **NBD fluorescence spectra were collected for each of the 24 NBD-labeled Cys residues of SecA.** The NBD-probe was covalently attached to the modified residue of SecA (see inset for location). NBD-fluorescence of SecA (1 μM) was then recorded between 510 and 560 nm using an excitation wavelength at 470 nm in the absence (black line) or presence (red line) of large unilamellar vesicles (LUVs, 2 mM) formed from *E. coli* lipids.

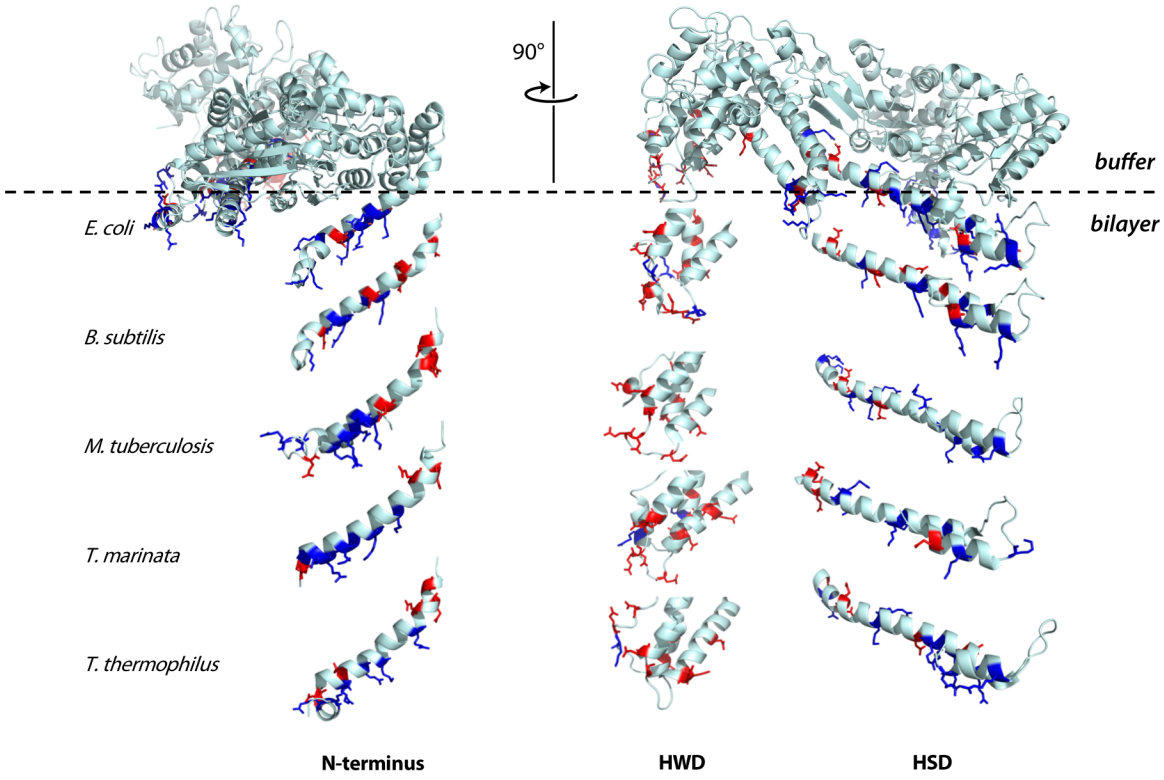

Figure S3 – Conserved surface charge distributions across species at the proposed membrane interface. *ec*SecA crystal structure with the positively (blue) and negatively (red) charged residues facing the proposed membrane location highlighted as sticks. For the different species, the charge distribution is conserved for all the three proposed membrane-interacting domains**.** Structures for *B. subtilis* (PDB: 1M6N; [57]), *M. tuberculosis* (PDB: 1NL3; [58]), *T. marinata* (PDB: 3JUX; [59]), and *T. thermophilus* (PDB: 2IPC; [60]). The structure of *E. coli* SecA was modeled after the *B. subtilis* structure determined by Hunt et al. [57], PDB:1M6N.

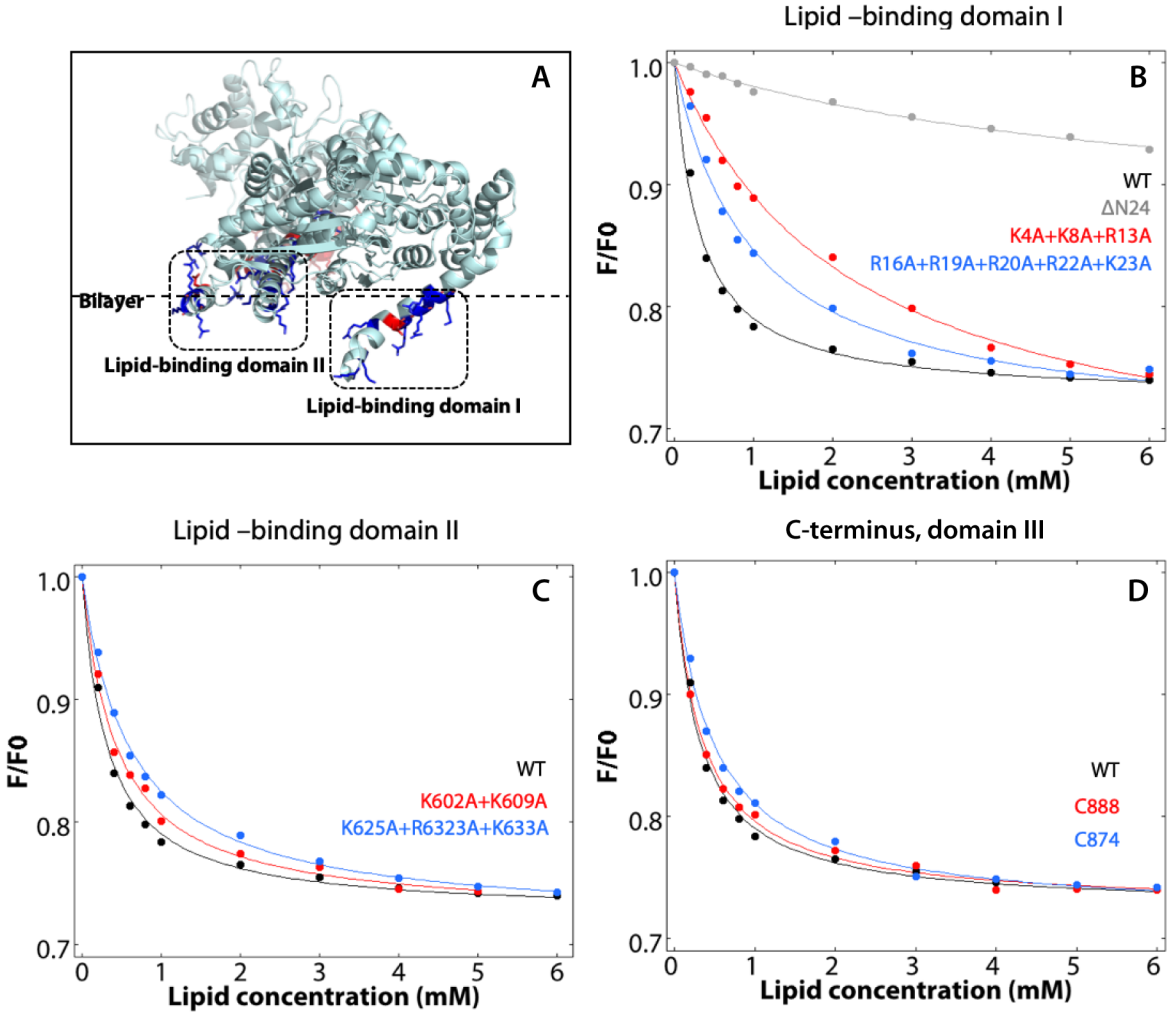

Figure S4 – Titration of SecA mutants with modified positive charge distributions in the proposed lipid-binding domains. A. *ec*SecA crystal structure with the positively (blue) and negatively (red) charged residues facing the membrane interface highlighted as sticks. The lipid-binding domains, labeled I and II, are shown in the dashed boxes. We also examined the relative binding importance of the C-terminus (defined as lipid binding domain III), which is not seen in any of the crystallographic structures. The relative importance of the charged residues in the three domains was determined from measurements of partition coefficients (Table I) by using relative tryptophan fluorescence intensity changes (F/F_0) accompanying the titration of aqueous solution of SecA (1 μM) with large unilamellar vesicles (LUV) made from *E. coli* lipids [53]. See Materials and Methods. Fluorescence intensities in the absence (F_0) or presence of lipid vesicles (F) were recorded between 310 and 400 nm using an excitation wavelength at 295 nm. For determination of F/F_0, we used the fluorescence intensities at 340 nm. **B.** SecA mutants were used to determine the relative importance of the positively charged residues in lipid-binding domain I: ΔN24 (the first

24 residues are removed; gray points), α0 (three alanine substitutions: K4A, K8A, and R13A; red points), and α1 (R16A, R19A, R20A, R22A, and K23A; blue points). **C.** Relative importance of the positively charged residues within lipid binding domain II: Residues located in the Joint domain (K602A+K609A, red) or residues located in the first half of the Helix Wing Domain (K625A+R632A+K633A). **D.** Key role of the positively charged residue in the lipid-binding domain III: The C-terminal tail of SecA was shortened by removing the residues after position 874 or position 888.

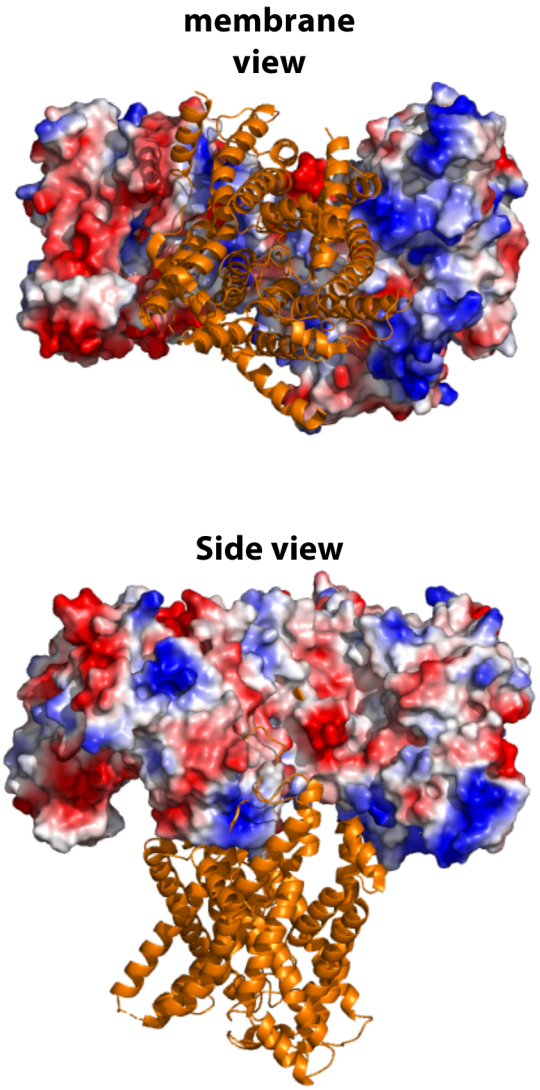

Figure S5 – Distribution of surface charges of SecA bound to the translocon. Left, cartoon representation of the SecA:SecY crystallographic complex (blue and yellow respectively, PDB code 5EUL, [61]). The electrostatic potential mapping (blue, positive charges; red, negative charges) on *Escherichia coli* SecA protein surface shows that SecA could bind to the membrane via its heavily positively charged N-terminus while bound to SecY. SecA could easily hop on-and-off SecY through negative-negative charges repulsion via the Helix Wing Domain, while the N-terminus remains attached to the membrane as proposed by Winkler *et al.* [62]

References

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