Supplementary Material

Supplementary discussion

Possible mechanisms for the propagation of conformational changes during the nucleotide hydrolysis cycle

We postulate that, during the transition from the ATP to the ADP state, both NBDs rotate outwards and towards the membrane. Because the PPXD is fixed by its interaction with the SecY channel, this could result in the transient widening of the clamp of SecA. The movement of the NBDs would also be propagated by the long helix of the HSD to the two-helix finger. In one scenario, the NBDs would maintain their interactions with the long helix of the HSD, resulting in its bending. This could change the tilt of the associated two-helix finger, such that its tip would move away from the SecY pore. An alternative possibility is that the interactions of the long helix of the HSD with the NBD1 are broken, perhaps by association of the NBD1 with a non-translocating SecY copy. In this case, the long helix would serve as a lever arm with a pivot point on the 2b/3 loop of SecY, and this could move the finger away from the SecY pore.

Supplementary figures

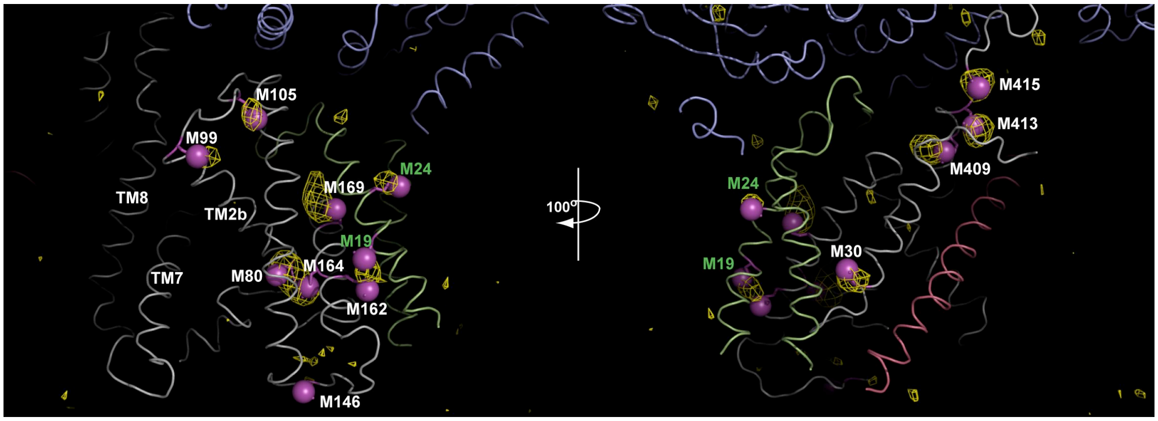

Figure S1| Position of the seleno-methionine residues in *T. maritima* SecYEG. The positions of 11 out of 13 Se-Met in SecY and SecG were identified in an anomalous Fourier difference map shown at 3.2σ contour level (yellow mesh). The positions for Se-Met 162 of SecY and 19 of SecG could not be assigned unambiguously. The sulfur atoms of all 13 Met residues of the SecYEG channel are shown as spheres in magenta. SecA is shown in blue, SecY in gray, SecE in red, and SecG in green.

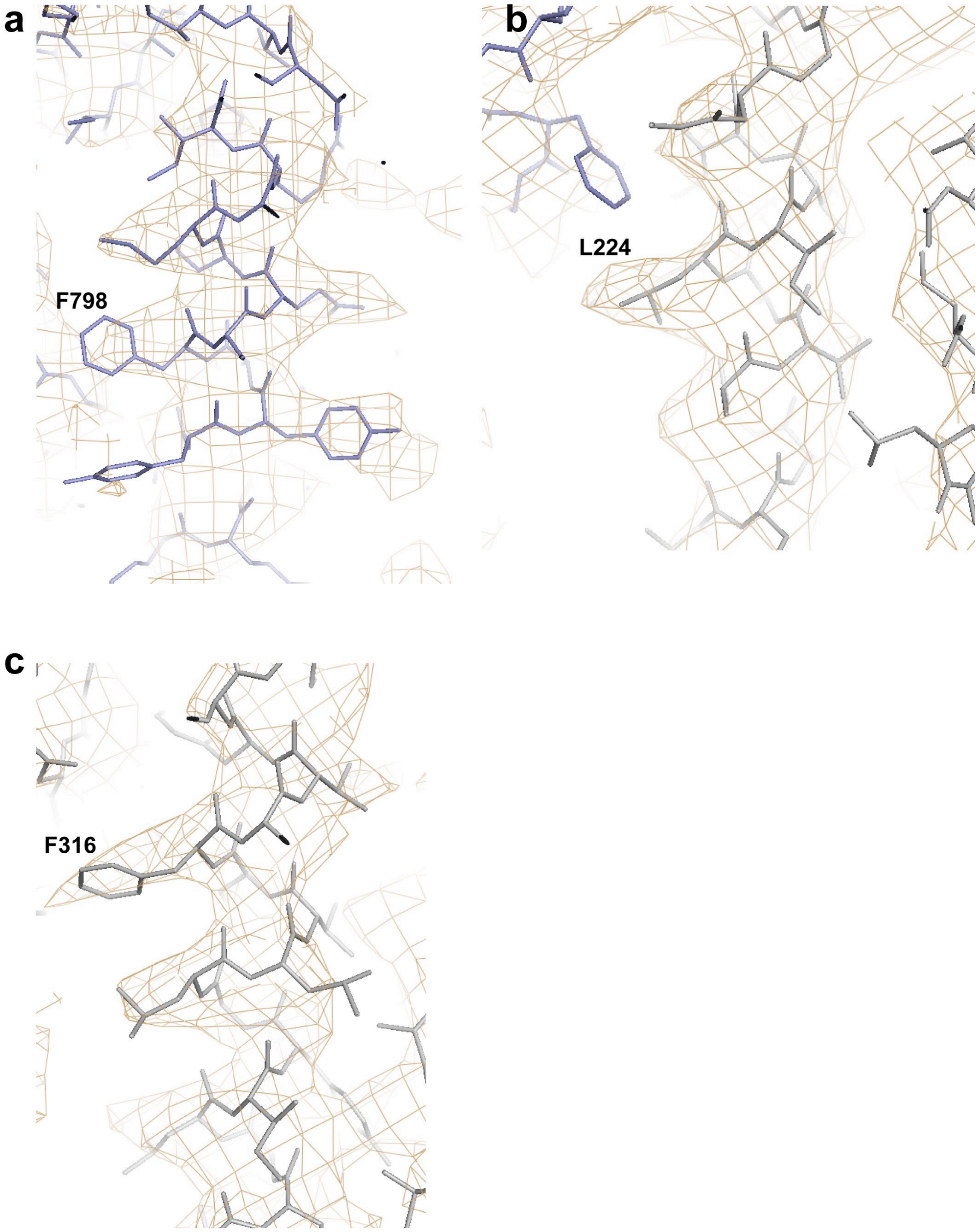

Figure S2| Selected views of the electron density map. Shown are σA-weighted, phase combined, NCS averaged, and B-factor sharpened (-100) 2FoFc electron density maps (brown mesh, contoured at 1σ). SecA is shown in blue and SecY in gray. **a**, Helix 2 of SecA's two helix finger inside the cytoplasmic funnel of SecY. **b** and **c**, TM6 and TM9 of SecY. Phe798 of SecA and Leu224 and Phe316 of SecY are labeled for orientation.

SecA

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E_coli          1 MLIKLLTKVFGSRNDRTLRRMRKVVNIINAMEPEMEKLSDEELKGKTAEFRARLEKGEVL
B_subtilis      1 -MLGILNKMFDP-TKRTLNRYEKIANDIDAIRGDYENLSDDALKHKTIEFKERLEKGATT
T_maritima      1 ------MILFDK-NKRILKKYAKMVSKINQIESDLRSKKNSELIRLSMVLKEKVNSFEDA

E_coli         61 ENLIPEAFAVVREASKRVFGMRHFDVQLLGGMVLNERCIAEMRTGEGKTLTATLPAYLNA
B_subtilis     59 DDLLVEAFAVVREASRRVTGMFPFKVQLMGGVALHDGNIAEMKTGEGKTLTSTLPVYLNA
T_maritima     54 DEHLFEAFALVREAARRTLGMRPFDVQVMGGIALHEGKVAEMKTGEGKTLAATMPIYLNA

E_coli        121 LTGKGVHVVTVNDYLAQRDAENNRPLFEFLGLTVGIN-----------------------
B_subtilis    119 LTGKGVHVVTVNEYLASRDAEQMGKIFEFLGLTVGLN-----------------------
T_maritima    114 LIGKGVHLVTVNDYLARRDALWMGPVYLFLGLRVGVINSLGKSYEVVWKNPDLARKAIEE

E_coli        158 ---------------LPGMPAPA------------KREAYAADITYGTNNEYGFDYLRDN
B_subtilis    156 ---------------LNSMSKDE------------KREAYAADITYSTNNELGFDYLRDN
T_maritima    174 NWSVWPDGFNGEVLKEESMNKEAVEAFQVELKEITRKEAYLCDVTYGTNNEFGFDYLRDN

E_coli        191 MAFSPEERVQRKLHYALVDEVDSILIDEARTPLIISGPAEDSSEMYKRVNKIIPHLIRQE
B_subtilis    189 MVLYKEQMVQRPLHFAVIDEVDSILIDEARTPLIISGQAAKSTKLYVQANAFVR------
T_maritima    234 LVLDYNDKVQRGHFYAIVDEADSVLIDEARTPLIISGPSKESPSVYRRFAQIAK------

E_coli        251 KEDSETFQGEGHFSVDEKSRQVNLTERGLVLIEELLVKEGIMDEGESLYSPANIMLMHHV
B_subtilis    243 -----TLKAEKDYTYDIKTKAVQLTEEGMTKAEKAFGID-------NLFDVKHVALNHHI
T_maritima    288 -----KFVKDKDFTVDEKARTIILTEEGVAKAEKIIGVE-------NLYDPGNVSLLYHL

E_coli        311 TAALRAHALFTRDVDYIVKDGEVIIVDEHTGRTMQGRRWSDGLHQAVEAKEGVQIQNENQ
B_subtilis    291 NQALKAHVAMQKDVDYVVEDGQVVIVDSFTGRLMKGRRYSEGLHQAIEAKEGLEIQNESM
T_maritima    336 INALKALHLFKKDVDYVVMNGEVIIVDEFTGRLLPGRRYSGGLHQAIEAKEGVPIKEESI

E_coli        371 TLASITFQNYFRLYEKLAGMTGTADTEAFEFSSIYKLDTVVVPTNRPMIRKDLPDLVYMT
B_subtilis    351 TLATITFQNYFRMYEKLAGMTGTAKTEEEEFRNIYNMQVVTIPTNRPVVRDDRPDLIYRT
T_maritima    396 TYATITFQNYFRMYEKLAGMTGTAKTEESEFVQVYGMEVVVIPTHKPMIRKDHDDLVFRT

E_coli        431 EAEKIQAIIEDIKERTAKGQPVLVGTISIEKSELVSNELTKAGIKHNVLNAKFHANEAAI
B_subtilis    411 MEGKFKAVAEDVAQRYMTGQPVLVGTVAVETSELISKLLKNKGIPHQVLNAKNHEREAQI
T_maritima    456 QKEKYEKIVEEIEKRYKKGQPVLVGTTSIEKSELLSSMLKKKGIPHQVLNAKYHEKEAEI

E_coli        491 VAQAGYPAAVTIATNMAGRGTDIVLGGSWQAEVAALENPTAEQIEKIKADWQVRHDAVLE
B_subtilis    471 IEEAGQKGAVTIATNMAGRGTDIKLG-----------------------------EGVKE
T_maritima    516 VAKAGQKGMVTIATNMAGRGTDIKLG-----------------------------PGVAE

E_coli        551 AGGLHIIGTERHESRRIDNQLRGRSGRQGDAGSSRFYLSMEDALMRIFASDRVSGMMRKL
B_subtilis    502 LGGLAVVGTERHESRRIDNQLRGRSGRQGDPGITQFYLSMEDELMRRFGAERTMAMLDRF
T_maritima    547 LGGLCIIGTERHESRRIDNQLRGRAGRQGDPGESIFFLSLEDDLLRIFGSEQIGKVMNIL

E_coli        611 GMKPGEAIEHPWVTKAIANAQRKVESRNFDIRKQLLEYDDVANDQRRAIYSQRNELLDVS
B_subtilis    562 GMDDSTPIQSKMVSRAVESSQKRVEGNNFDSRKQLLQYDDVLRQQREVIYKQRFEVIDSE
T_maritima    607 KIEEGQPIQHPMLSKLIENIQKKVEGINFSIRKTLMEMDDVLDKQRRAVYSLRDQILLEK

E_coli        671 DVSETINSIREDVFKATIDAYIPPQSLEEMWDIPGLQERLKNDFDLD--LPIAEWLDKEP
B_subtilis    622 NLREIVENMIKSSLERAIAAYTPREELPEEWKLDGLVDLINTTYLDEGALEKSDIFGKEP
T_maritima    667 DYDEYLKDIFEDVVSTRVEEFCSGK----NWDIESLKNSLS--FFPAGLFDLDEKQFSSS

E_coli        729 ELHEETLRERILAQSIEVYQRKEEVVGAEMMRHFEKGVMLQTLDSLWKEHLAAMDYLRQG
B_subtilis    682 ----DEMLELIMDRIITKYNEKEEQFGKEQMREFEKVIVLRAVDSKWMDHIDAMDQLRQG
T_maritima    721 ----EELHDYLFNRLWEEYQRKKQEIG-EDYRKVIRFLMLRIIDDHWRRYLEEVEHVKEA

E_coli        789 IHLRGYAQKDPKQEYKRESFSMFAAMLESLKYEVISTLSKVQVRMPEEVEELEQQRRMEA
B_subtilis    738 IHLRAYAQTNPLREYQMEGFAMFEHMIESIEDEVAKFVMKAEIENNLEREEVVQG-----
T_maritima    776 VQLRSYGQKDPIVEFKKETYYMFDEMMRRINDTIANYVLRVVKVSEKDEKEAKEELG---

E_coli        849 ERLAQMQQLSHQDDDSAAAAALAAQTGERKVGRNDPCPCGSGKKYKQCHGRLQ
B_subtilis    793 ----QTTAHQPQEGDDNKKAKKAPVRKVVDIGRNAPCHCGSGKKYKNCCGRTE
T_maritima    833 -----KIRLVHEEFNLVNRAMRRATEKKK---KKDGLHSFGRIRVKR------
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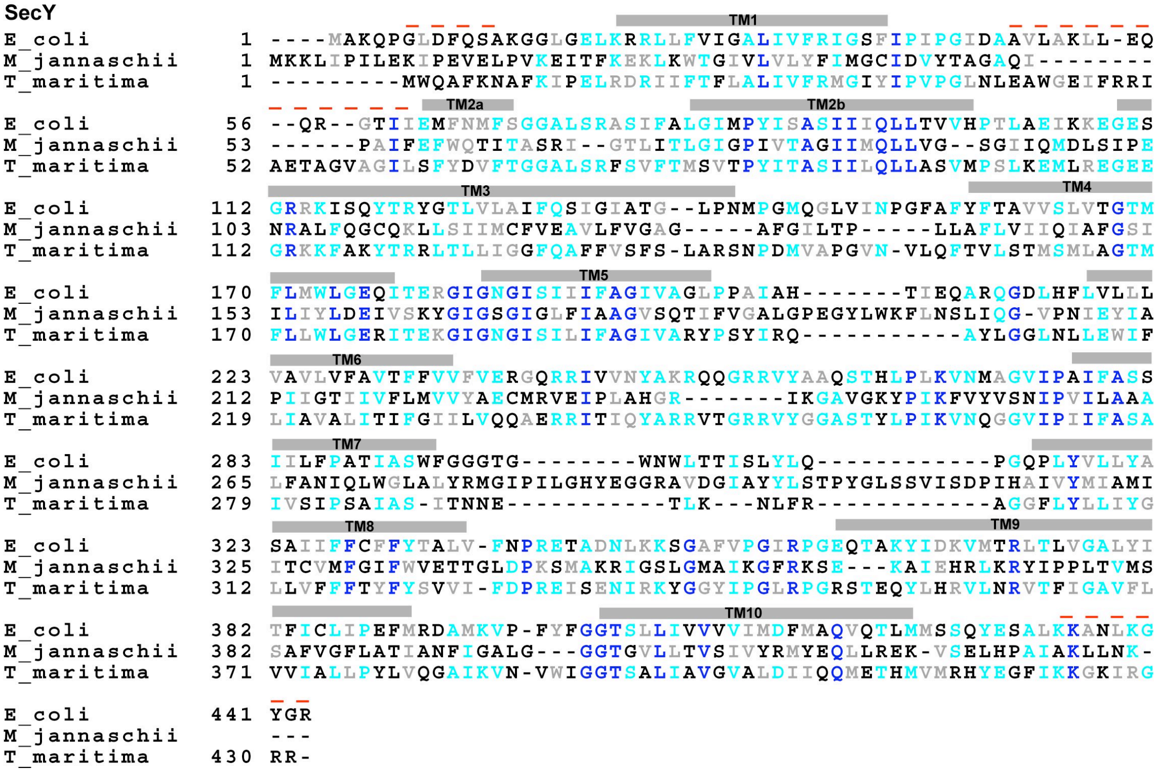
Figure S3| Sequence alignment of the SecAs from *E. coli, B. subtilis and T. maritima*. The C-termini of the truncated SecA constructs used in this study are indicated by a red bar.



```
E_coli           1 ----MAKQPGLDFQSAKGGLGELKRRLLFVIGALIVFRIGSFIPIPGIDAAVLAKLL-EQ
M_jannaschii     1 MKKLIPILEKIPEVELPVKEITFKEKLKWTGIVLVLYFIMGCIDVYTAGAQI--------
T_maritima       1 ---------MWQAFKNAFKIPELRDRIIFTFLALIVFRMGIYIPVPGLNLEAWGEIFRRI

E_coli          56 --QR--GTIIEMFNMFSGGALSRASIFALGIMPYISASIIIQLLTVVHPTLAEIKKEGES
M_jannaschii    53 ------PAIFEFWQTITASRI--GTLITLGIGPIVTAGIIMQLLVG--SGIIQMDLSIPE
T_maritima      52 AETAGVAGILSFYDVFTGGALSRFSVFTMSVTPYITASIILQLLASVMPSLKEMLREGEE

E_coli         112 GRRKISQYTRYGTLVLAIFQSIGIATG--LPNMPGMQGLVINPGFAFYFTAVVSLVTGTM
M_jannaschii   103 NRALFQGCQKLLSIIMCFVEAVLFVGAG-----AFGILTP-----LLAFLVIIQIAFGSI
T_maritima     112 GRKKFAKYTRRLTLLIGGFQAFFVSFS-LARSNPDMVAPGVN-VLQFTVLSTMSMLAGTM

E_coli         170 FLMWLGEQITERGIGNGISIIIFAGIVAGLPPAIAH-------TIEQARQGDLHFLVLLL
M_jannaschii   153 ILIYLDEIVSKYGIGSGIGLFIAAGVSQTIFVGALGPEGYLWKFLNSLIQG-VPNIEYIA
T_maritima     170 FLLWLGERITEKGIGNGISILIFAGIVARYPSYIRQ-----------AYLGGLNLLEWIF

E_coli         223 VAVLVFAVTFFVVFVERGQRRIVVNYAKRQQGRRVYAAQSTHLPLKVNMAGVIPAIFASS
M_jannaschii   212 PIIGTIIVFLMVVYAECMRVEIPLAHGR-------IKGAVGKYPIKFVYVSNIPVILAAA
T_maritima     219 LIAVALITIFGIILVQQAERRITIQYARRVTGRRVYGGASTYLPIKVNQGGVIPIIFASA

E_coli         283 IILFPATIASWFGGGTG-------WNWLTTISLYLQ-----------PGQPLYVLLYA
M_jannaschii   265 LFANIQLWGLALYRMGIPILGHYEGGRAVDGIAYYLSTPYGLSSVISDPIHAIVYMIAMI
T_maritima     279 IVSIPSAIAS-ITNNE-----------TLK---NLFR------------AGGFLYLLIYG

E_coli         323 SAIIFFCFFYTALV-FNPRETADNLKKSGAFVPGIRPGEQTAKYIDKVMTRLTLVGALYI
M_jannaschii   325 ITCVMFGIFWVETTGLDPKSMAKRIGSLGMAIKGFRKSE---KAIEHRLKRYIPPLTVMS
T_maritima     312 LLVFFFTYFYSVVI-FDPREISENIRKYGGYIPGLRPGRSTEQYLHRVLNRVTFIGAVFL

E_coli         382 TFICLIPEFMRDAMKVP-FYFGGTSLLIVVVVIMDFMAQVQTLMMSSQYESALKKANLKG
M_jannaschii   382 SAFVGFLATIANFIGALG---GGTGVLLTVSIVYRMYEQLLREK-VSELHPAIAKLLNK-
T_maritima     371 VVIALLPYLVQGAIKVN-VWIGGTSALIAVGVALDIIQQMETHMVMRHYEGFIKKGKIRG

E_coli         441 YGR
M_jannaschii       ---
T_maritima     430 RR-
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Figure S4| Sequence alignment of the SecYs from *E. coli, M. jannaschii and T. maritima*. TM-helices are indicated by gray bars, and regions not modeled in our crystal structure are indicated by red dashed lines.

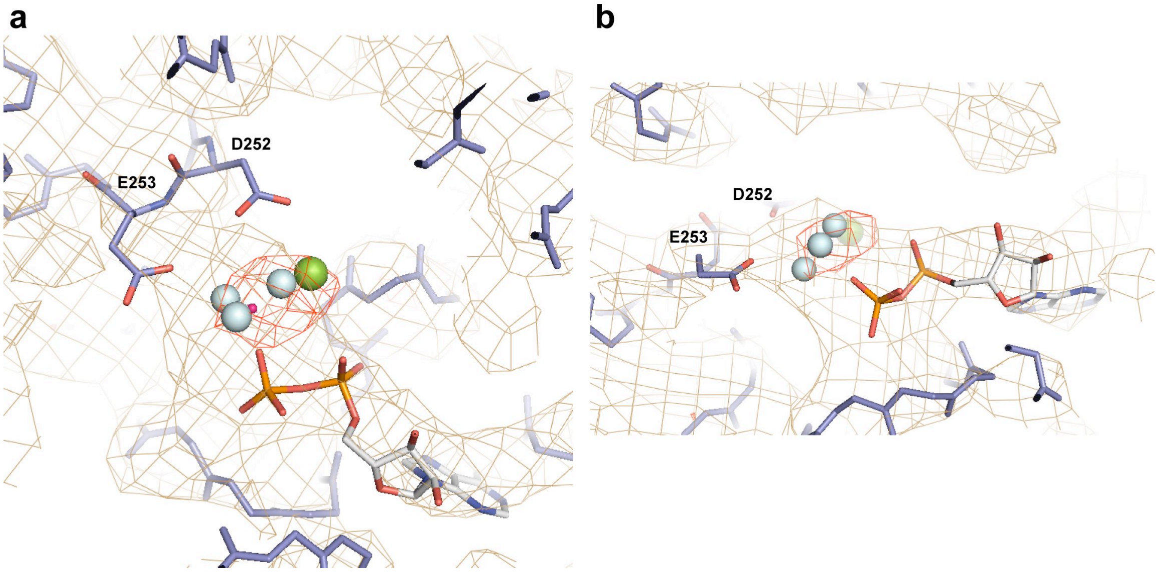


Figure S5| The ADP-BeFx complex in the nucleotide binding pocket of SecA. **a**, A σA-weighted, phase combined, NCS averaged, and B-factor sharpened (-50) 2FoFc electron density map (brown mesh, contoured at 1.2σ). The model phases were calculated with only ADP in the nucleotide-binding pocket and were also used to calculate a FoFc difference map (red mesh, contoured at 4.5σ). The modeled Mg^{2+}/BeF_3^- complex is shown in spheres (green magnesium, blue fluorine, pink beryllium). **b**, as in **a**, but side view of the ADP-BeF_3^- complex. Asp252 and Glu253 in the DEAD box of SecA are labeled for orientation.

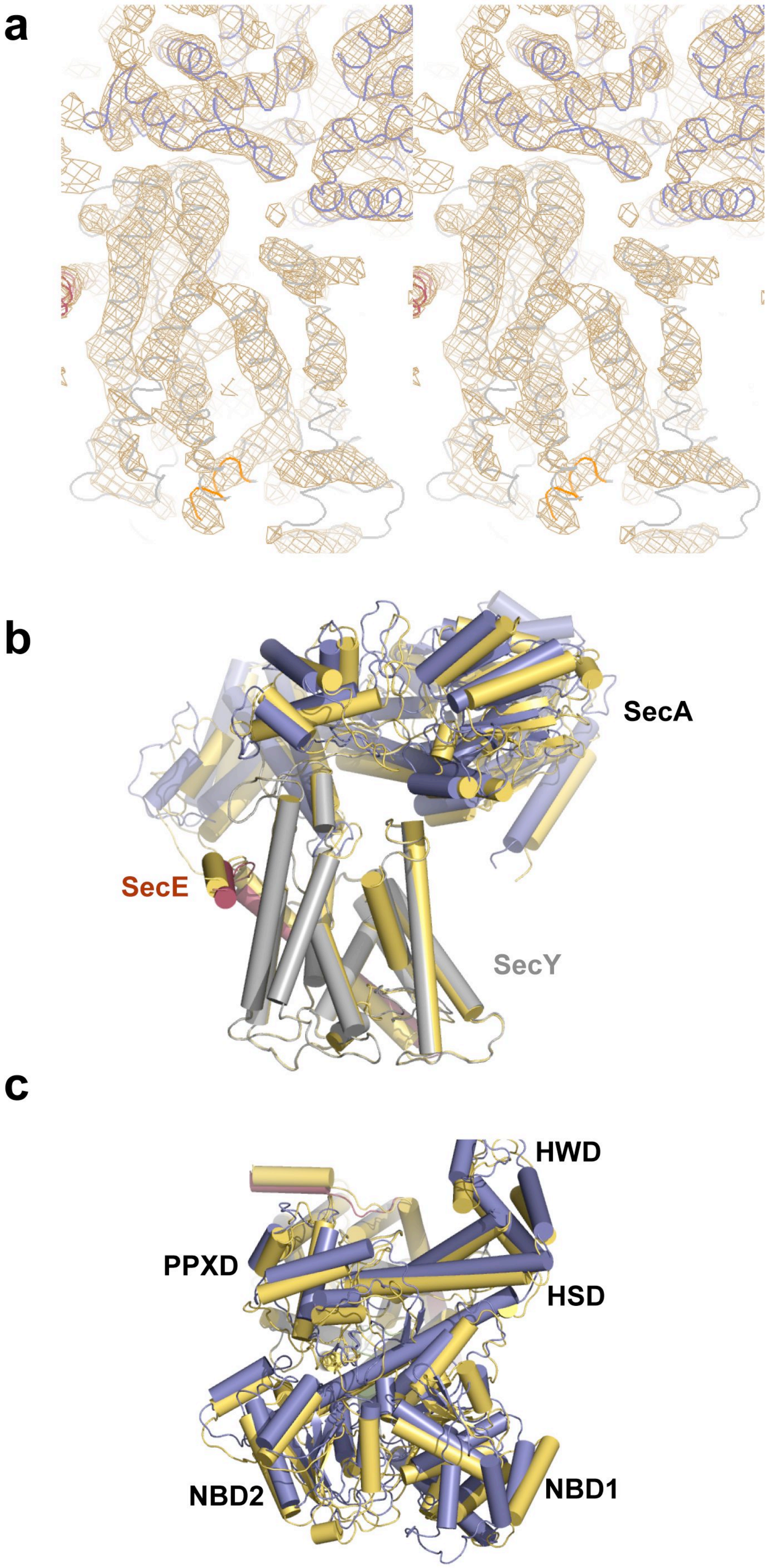

a
b
SecA
SecE
SecY
c
HWD
PPXD
HSD
NBD2
NBD1

Figure S6| Comparison of the *B. subtilis* SecA – *T. maritima* SecYE and *T. maritima* SecA-SecYEG complexes. **a**, Stereo view of a σA-weighted, 4-fold averaged, solvent-flattened 2FoFc electron density map of the *B. subtilis* SecA - *T. maritima* SecYE complex. The view is on the lateral gate of SecY. SecY is shown in gray, the plug domain in orange, and SecA in blue. **b**, Side view of an alignment of the complex of *B. subtilis* SecA and *T. maritima* SecYE (both components in yellow) with the complex of *T. maritima* SecA and *T. maritima* SecYEG (SecA in blue, SecY in gray, SecE in red, SecG omitted). The complexes were aligned with respect to the SecY channel. **c**, Top view of the aligned complexes.

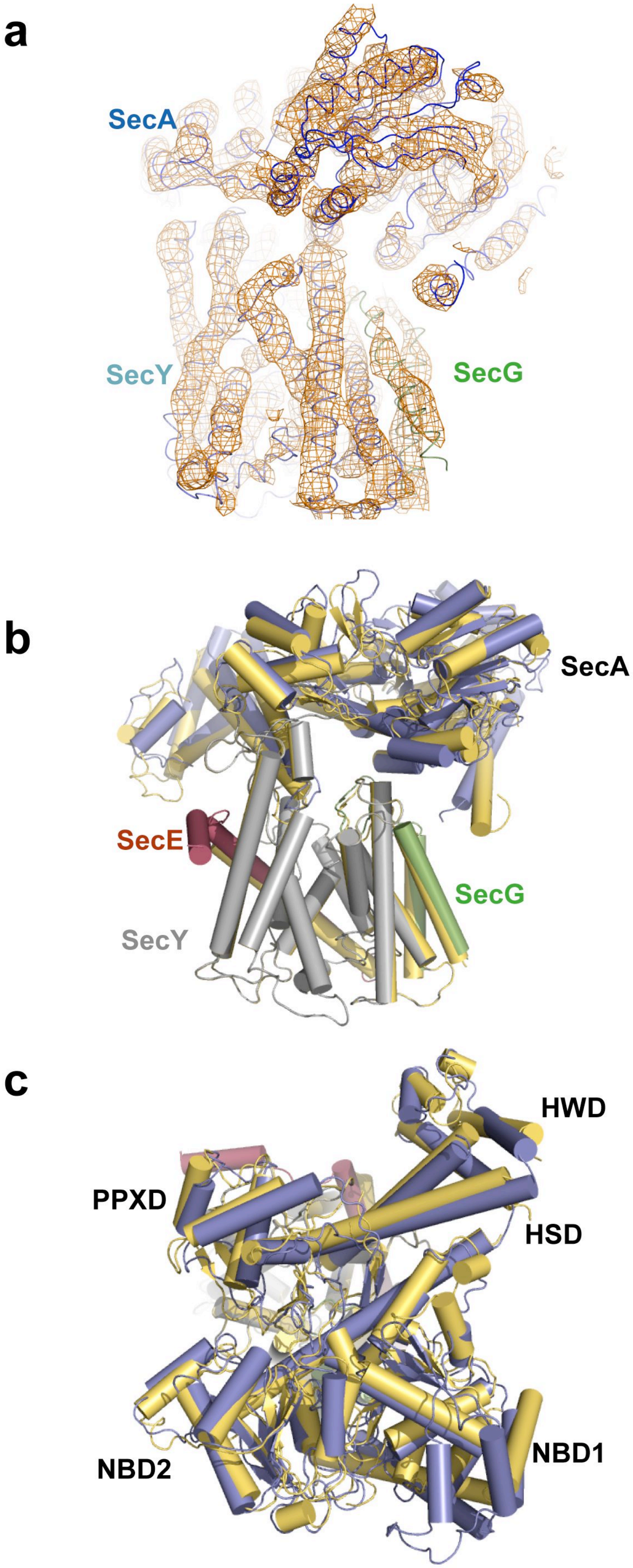

a
SecA
SecY
SecG
b
SecA
SecE
SecG
SecY
c
HWD
PPXD
HSD
NBD2
NBD1

Figure S7| Comparison of the *B. subtilis* SecA – *A. aeolicus* SecYEG and *T. maritima* SecA-SecYEG complexes. **a**, Shown is a NCS averaged 2FoFc electron density map of the *B. subtilis* SecA - *A. aeolicus* SecYEG complex. The view is on the lateral gate of SecY. SecY is shown in light blue, SecG in green, SecE in red, and SecA in blue. **b**, Side view of an alignment of the complex of *B. subtilis* SecA and *A. aeolicus* SecYEG (both components in yellow) with the complex of *T. maritima* SecA and *T. maritima* SecYEG (SecA in blue, SecY in gray, SecE in red, SecG green). The complexes were aligned with respect to the SecY channel. **c**, Top view of the aligned complexes.

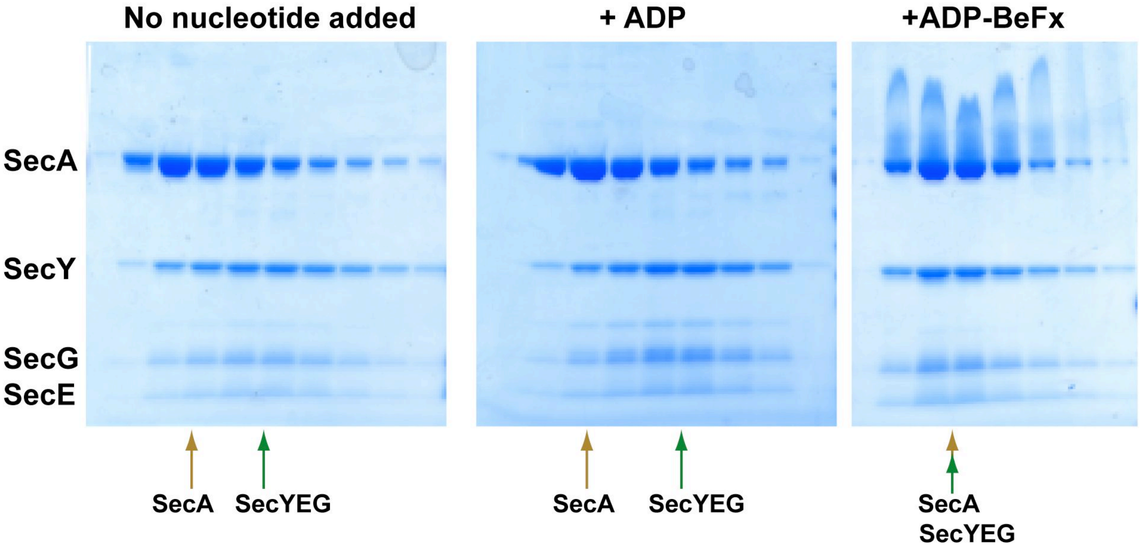


Figure S8| Nucleotide dependence of the stability of a SecA-SecY complex. SecA from *B. subtilis* and SecYEG from *A. aeolicus* were gel-filtered in the absence of nucleotide or in the presence of ADP or ADP-BeFx in 20mM MES pH 6.5, 150mM NaCl, 10% glycerol, 0.5mM Cymal-6, 10mM $MgCl_2$ (± 1mM ADP, 2mM $BeCl_2$, and 7mM NaF). Samples of the fractions were analyzed by SDS-PAGE and stained with Coomassie blue. The "smearing" of SecA in the presence of ADP and BeFx is likely due to an increased SDS resistance of SecA and disappears upon boiling of the samples in SDS prior to electrophoresis. The arrows indicate the peak positions of SecA and SecY.

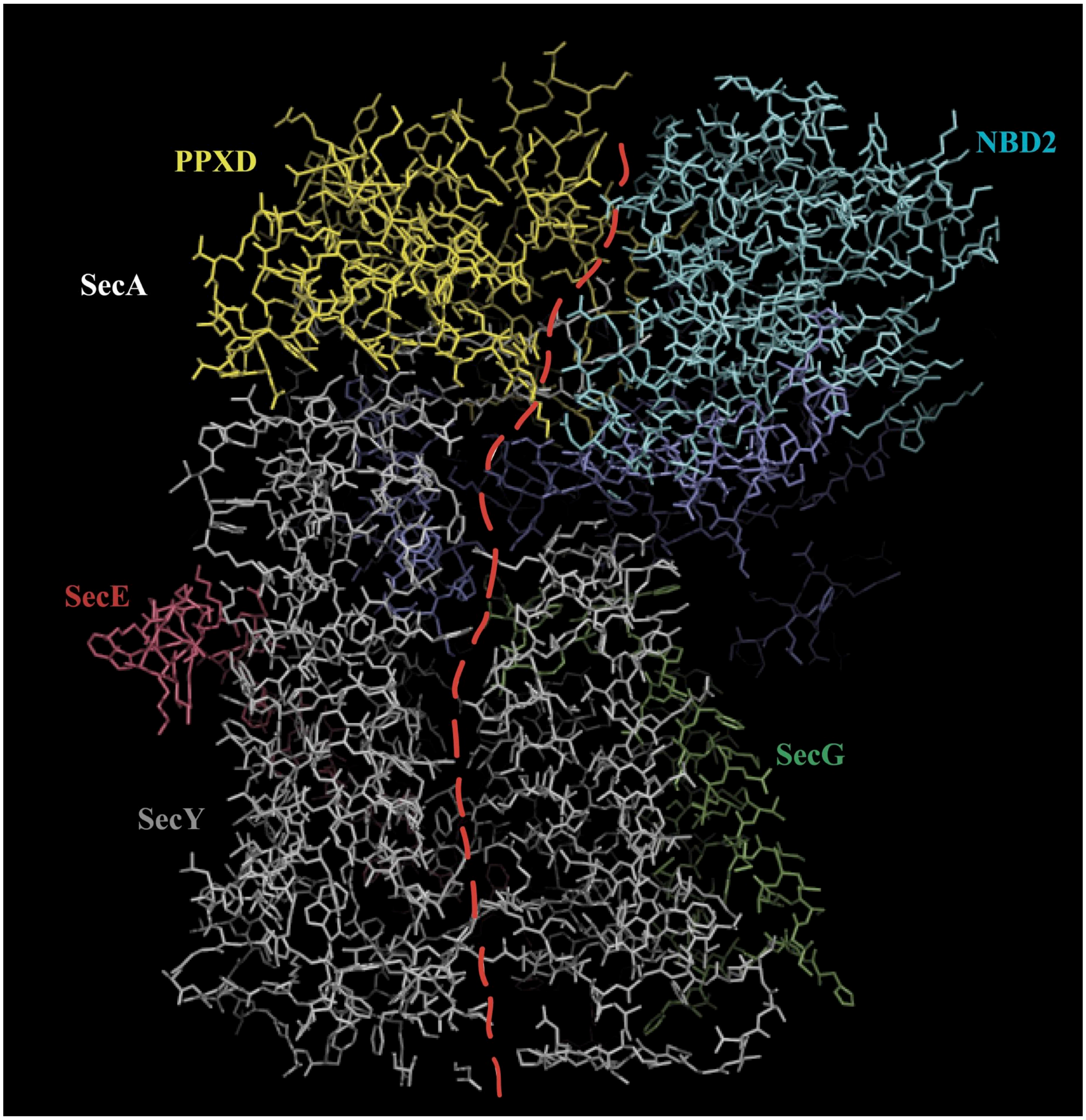


Figure S9| Alignment of the mouth of the SecA clamp with the lateral gate of the SecY channel. The seam on the surface of the SecA-SecYEG complex is indicated by a dashed red line. The polypeptide chains are colored as in Figure 1.

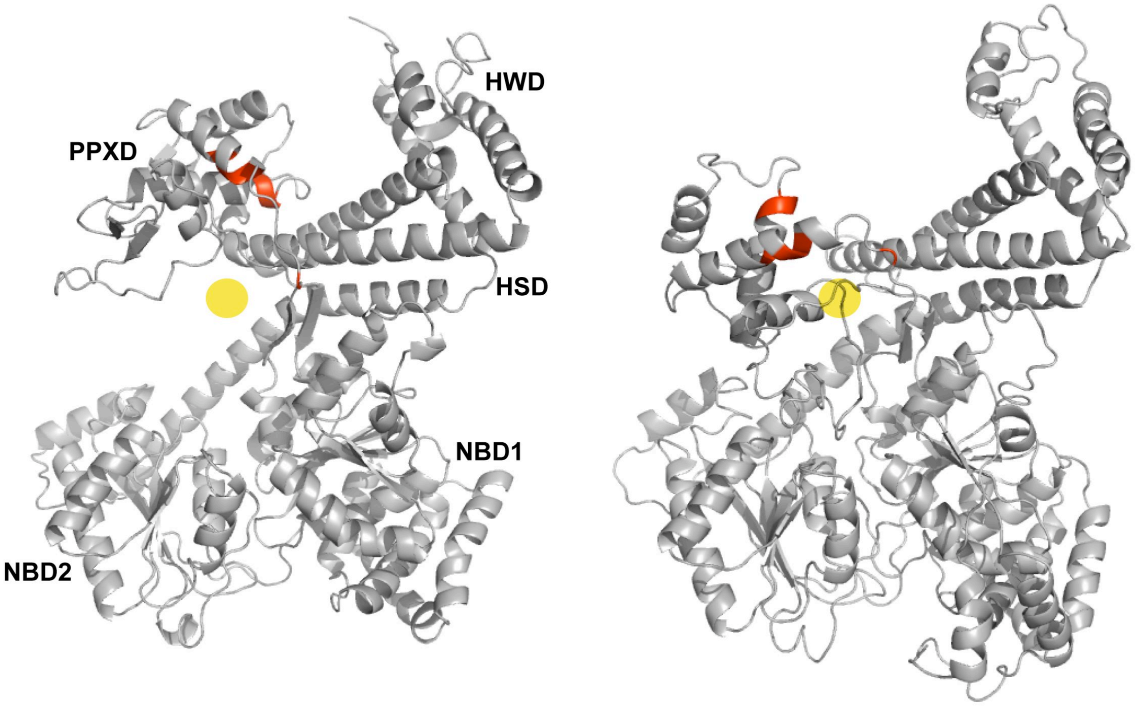
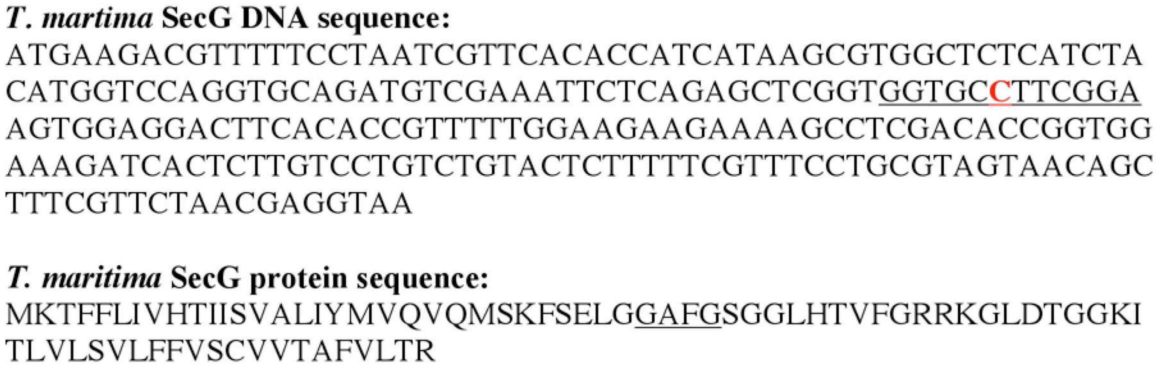


Figure S10| Signal peptide binding site of SecA as identified by NMR spectroscopy. SecA residues interacting with the hydrophobic core of the synthetic signal peptide KRR-LamB are mapped in red onto *B. subtilis* SecA (left) and the *T. maritima* SecA-SecYEG complex (right) (*E. coli* residues: P228, M235, Y236, V239, I304, M305, L306). The position of the SecY pore is indicated by a yellow circle.



***T. martima* SecG DNA sequence:**
ATGAAGACGTTTTTCCTAATCGTTCACACCATCATAAGCGTGGCTCTCATCTA
CATGGTCCAGGTGCAGATGTCGAAATTCTCAGAGCTCGGTGGTGC**C**TTCGGA
AGTGGAGGACTTCACACCGTTTTTGGAAGAAGAAAAGCCTCGACACCGGTGG
AAAGATCACTCTTGTCCTGTCTGTACTCTTTTTCGTTTCCTGCGTAGTAACAGC
TTTCGTTCTAACGAGGTAA

***T. maritima* SecG protein sequence:**
MKTFFLIVHTIISVALIYMVQVQMSKFSELGGAFGSGGLHTVFGRRKGLDTGGKI
TLVLSVLFFVSCVVTAFVLTR

Figure S11| Nucleotide and amino acid sequence of *T. maritima* SecG. The gene was PCR-amplified from genomic DNA (ATCC code 43589, DSM: 3109). The database entry for the *T. maritima* SecG gene has a nucleotide deletion after the Ala33 codon, resulting in a frame shift. The extra cytidine found in our sequence is highlighted in red.

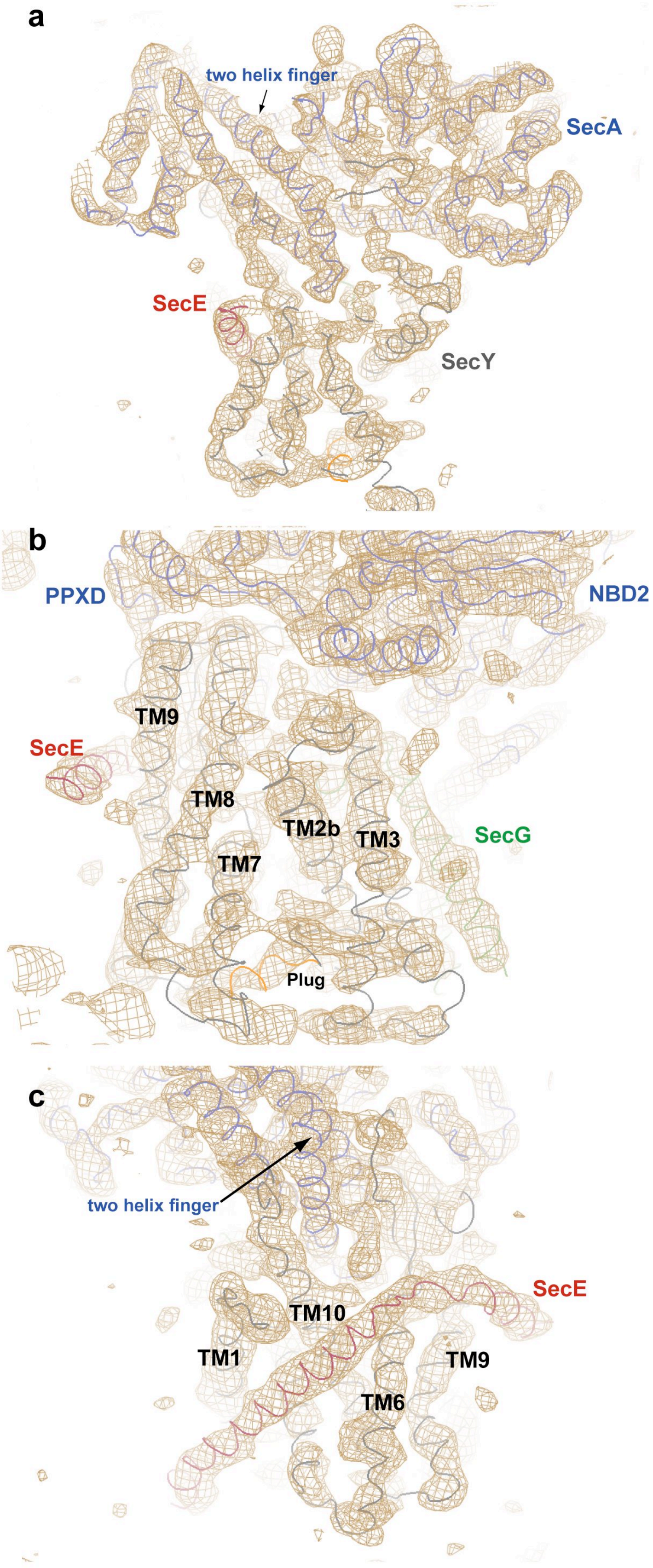

a
two helix finger
SecA
SecE
SecY
b
PPXD
NBD2
TM9
SecE
TM8
TM2b
TM3
SecG
TM7
Plug
c
two helix finger
SecE
TM10
TM1
TM9
TM6

Figure S12| Experimental electron density map for the *T. maritima* SecA-SecYEG complex. The map was calculated at 4.5Å and contoured at 1.0σ. Experimental phases were derived from 11 Se-Met positions identified in SecY and SecG in an anomalous Fourier difference map and were refined by NCS averaging. **a**, Overview of the SecA-SecYEG complex; **b**, Close-up view on the lateral gate of SecY. **c**, 90° rotation of **b**. SecA is shown in blue, SecY in gray, SecE in red, and SecG in green. SecY's plug is shown in orange.