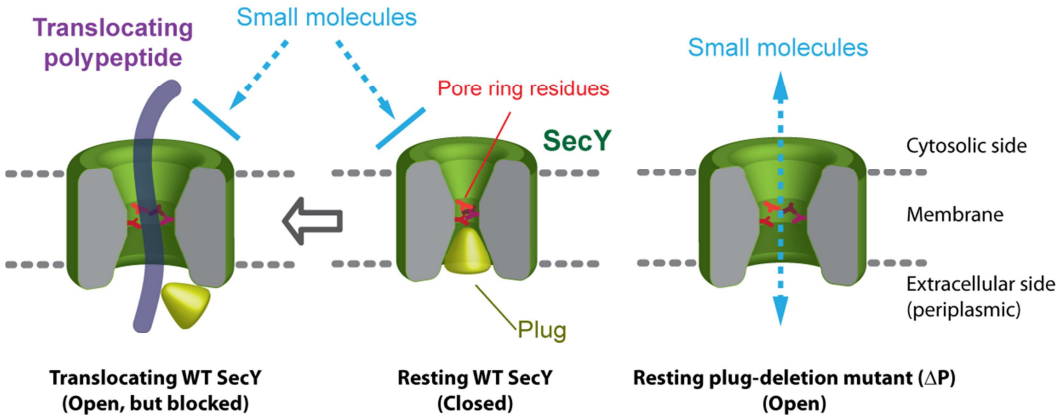
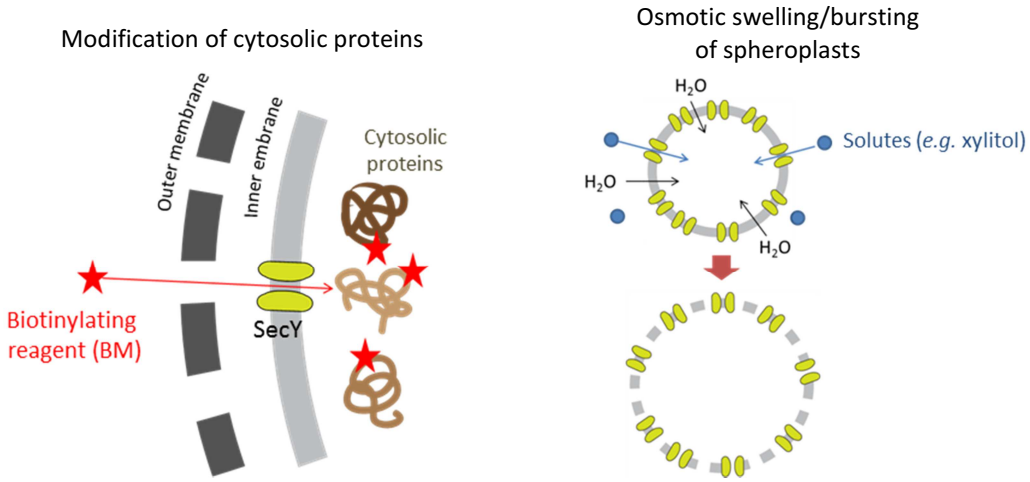
SUPPLEMENTARY FIGURES

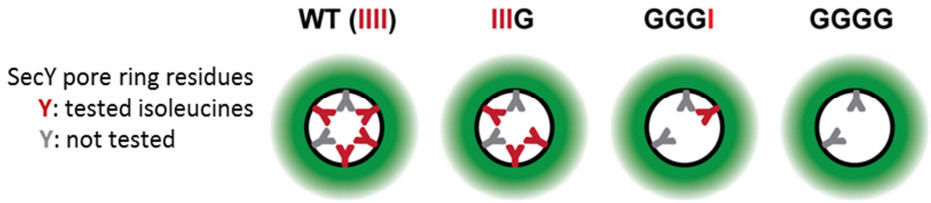
a. Models for the resting and translocating SecY Channel



b. Methods to determine permeability of the SecY channel for small molecules



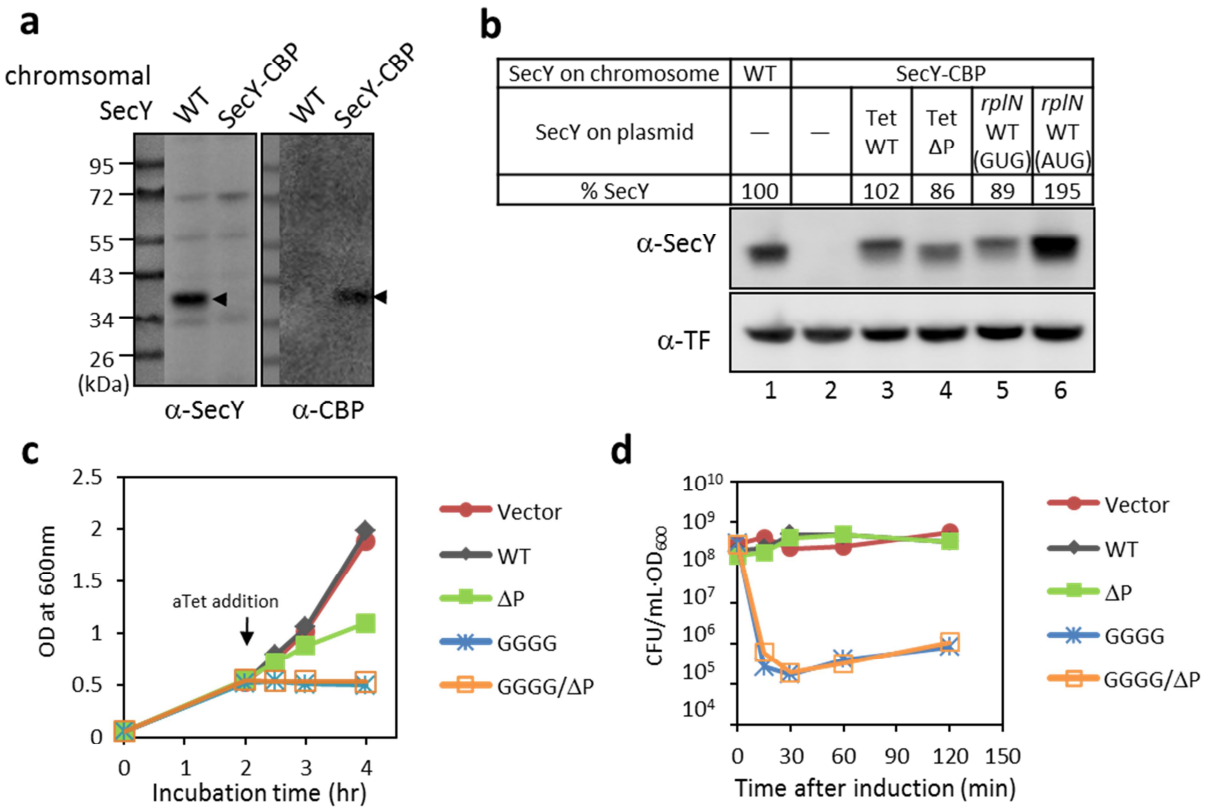
c. Permeability of pore mutants



Permeation of	- NC	+ NC	- NC	+ NC	- NC	+ NC	- NC	+ NC
BM (525 Da)	—	—	-/+	—	++	—	++	—
Xylitol (152 Da)	—	—	+	—	++	++	n.d.	n.d.
Cl^- (35 Da)	—	—	+	+	++	++	n.d.	n.d.

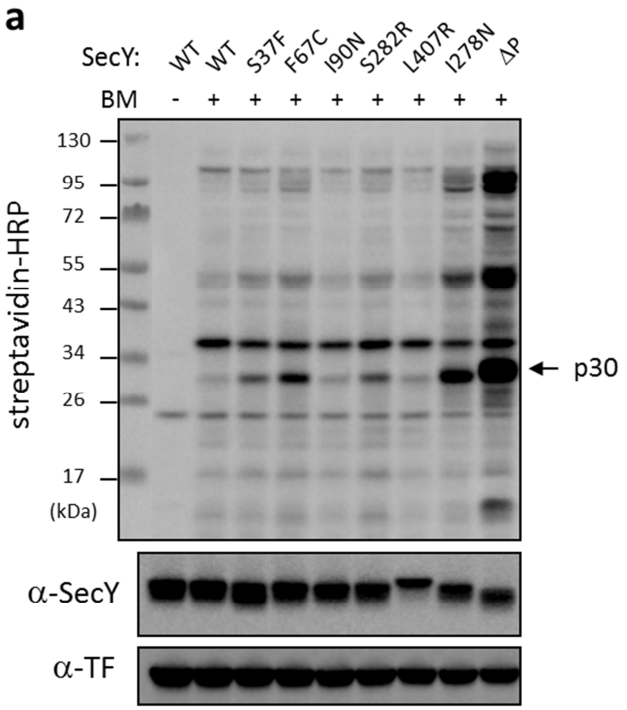
Permeation: —, none; -/+, little; +, moderate; ++, high; n.d., not determined
NC: translocating nascent chain in the pore

Supplementary Figure 1 | Summarizing figure



Supplementary Figure 2 | Expression level of SecY and growth properties of cells expressing wild-type SecYs or its mutants.

a, Wild-type (WT) cells (*Δrmf ΔompT*; EP51) and cells containing an endogenous SecY copy on the chromosome with a calmodulin-binding peptide (CBP) tag at the C-terminus (*Δrmf ΔompT secY-CBP*; EP52) were analyzed by SDS-PAGE and immunoblotting with SecY- or CBP-antibodies. The arrowheads indicate the position of SecY. CBP-tagging at the C-terminus of SecY abolishes recognition of SecY by SecY antibodies. **b**, SecY expression from plasmids was compared with that of chromosomal SecY in wild-type cells (EP51; lane 1) by immunoblotting with SecY antibodies. Wild-type or ΔP SecY were expressed from the inducible Tet promoter (induced for 30 min from pTet-SecYEG; lane 3 and 4) or the constitutive endogenous *rplN* promoter (pACYC-SecYEG; lane 5 and 6). Where indicated, the start-codon for SecY was changed to GUG (lane 5). The same amount of cell lysates was loaded in each lane. Quantification was done by densitometry of SecY bands. **c**, Growth of cells expressing wild-type SecY, the ΔP mutant, a pore mutant with four glycine substitutions (GGGG), or a mutant containing both mutations (GGGG/ΔP). Where indicated, anhydrotetracycline (aTet) was added to express these proteins from the Tet promoter. **d**, At various times after addition of aTet to the cell cultures, the number of colony-forming units (CFU) was determined by plating serial dilutions of the cells.

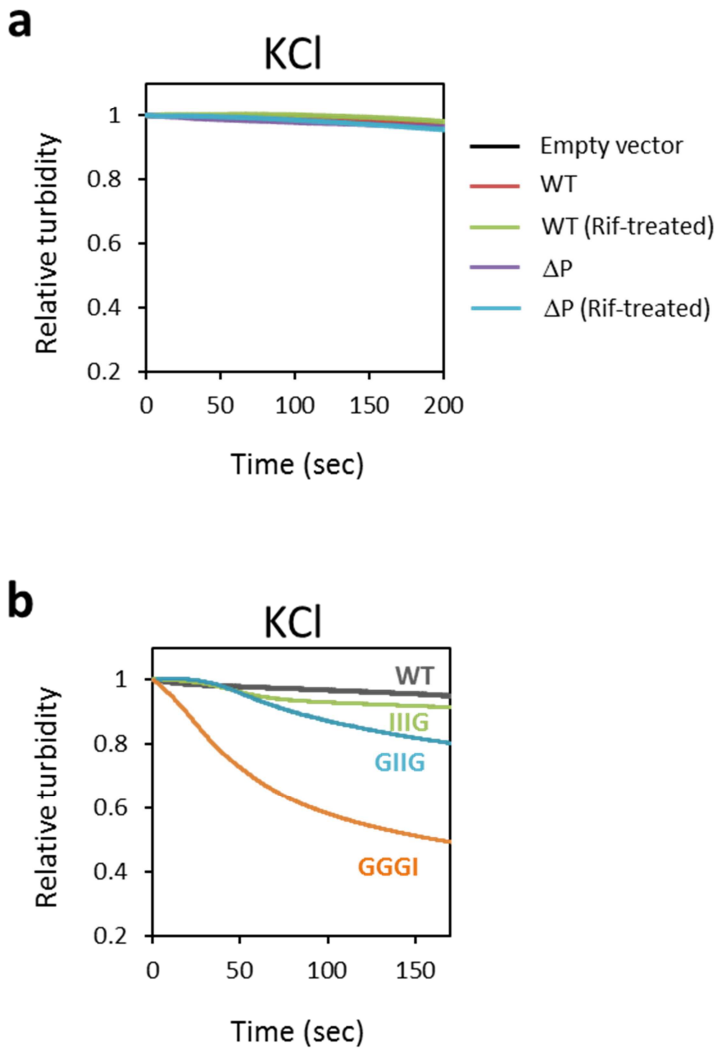


b

Densitometry of p30 band	
WT	5%
S37F	12%
F67C	21%
I90N	6%
S282R	13%
L407R	7%
I278N	36%
ΔP	100%

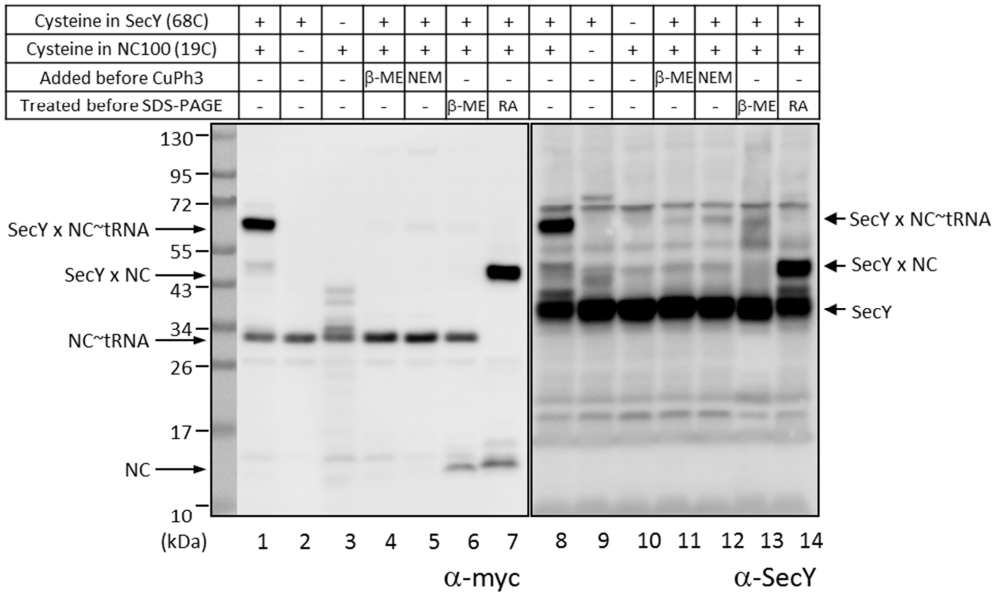
Supplementary Figure 3 | Permeability of signal sequence suppressor SecY mutants for the modification reagent BM.

a, EP52 cells expressing wild-type (WT) SecY or the indicated mutants from pTet-SecYEG were incubated with BM. The samples were analyzed by SDS-PAGE followed by blotting with streptavidin-HRP conjugate or SecY- and TF- antibodies. The same amount of cell lysates was loaded in each lane. **b**, Quantification of modified p30. The intensity of the band is given relative to that seen with a plug-deletion mutant (ΔP).



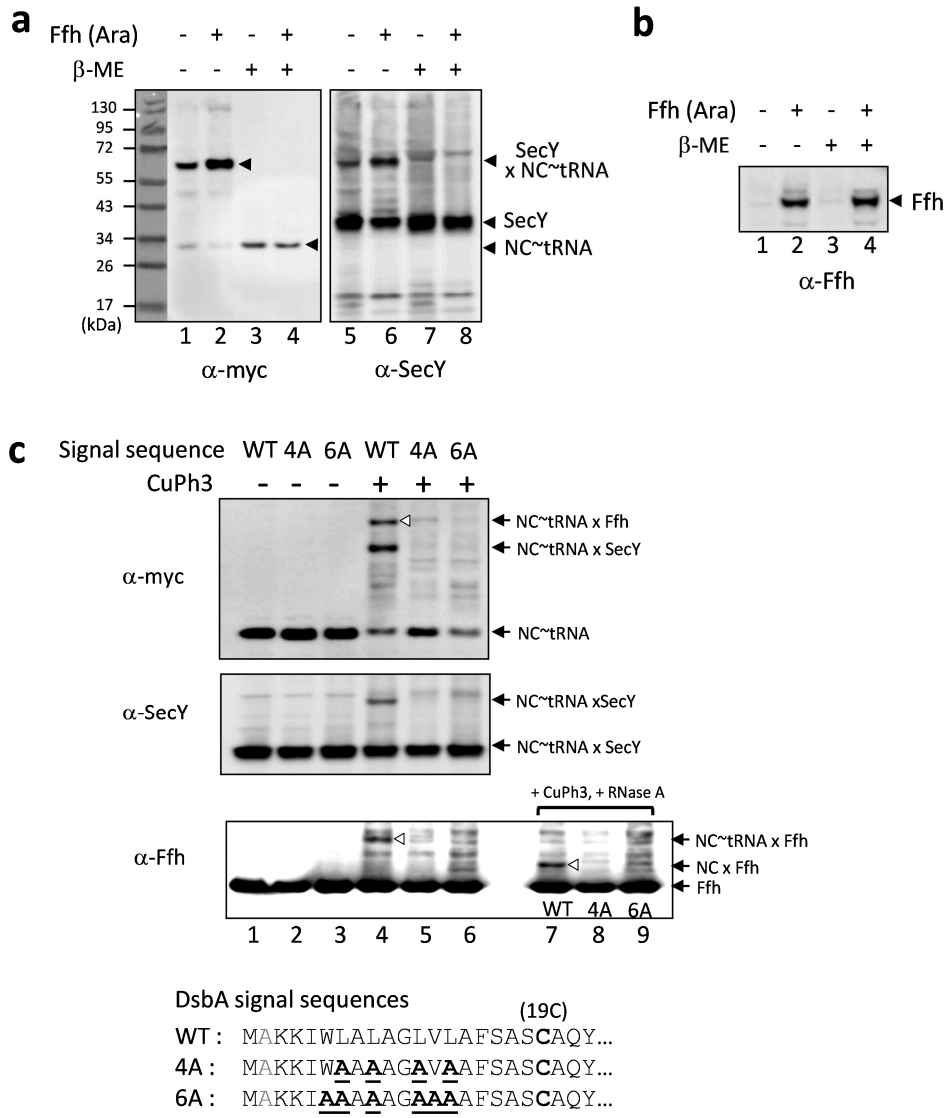
Supplementary Figure 4 | Permeability of wild-type and mutant SecY for KCl.

a, Spheroplasts of *Δrmf ΔompT secY-CBPΔglpF* (EP53) cells expressing wild-type (WT) SecY or a plug-deletion (ΔP) mutant under the Tet promoter (pTet-SecYEG) were diluted into an iso-osmotic solution of KCl in the absence of valinomycin. The osmotic swelling/bursting of the cells was followed by measuring the turbidity over time. Where indicated, rifampicin (Rif) was added before spheroplasting. In these experiments, osmotic swelling/bursting requires permeation of both K^+ and Cl^- into spheroplasts, driven by a high concentration of KCl in the medium, since movement of K^+ or Cl^- alone is inhibited by the generation of a membrane potential. **b**, As in **a**, but pore mutants with different numbers of glycine substitutions were tested.



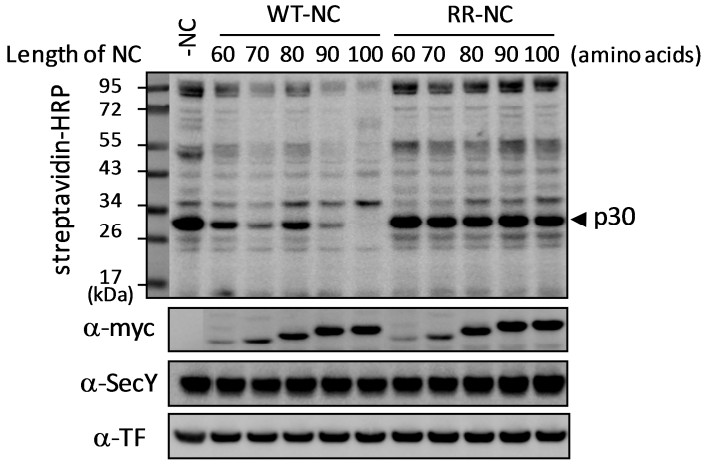
Supplementary Figure 5 | Generation of a translocation intermediate *in vivo*

NC100 with or without Cys19 was expressed for 2 hrs in EP51 cells expressing SecY with or without Cys68 under the endogenous promoter (from pRSY). Intact cells were treated with the oxidant copper phenanthroline (CuPh3) to induce disulfide bridge formation and analyzed by non-reducing SDS-PAGE and immunoblotting with myc- or SecY- antibodies. Where indicated, 30 mM of β-mercaptoethanol (β-ME; lane 4 and 11) or *N*-ethylmaleimide (NEM; lane 5 and 12) was added into the cell cultures before CuPh3 to prevent crosslinking. The sample in lane 1 and 8 was treated with β-mercaptoethanol (β-ME; lanes 6 and 13) or with RNase A (RA; lane 7 and 14) prior to SDS-PAGE. SecY x NC~tRNA and SecY x NC indicate SecY crosslinks of nascent chains (NC) linked to or lacking tRNA, respectively.



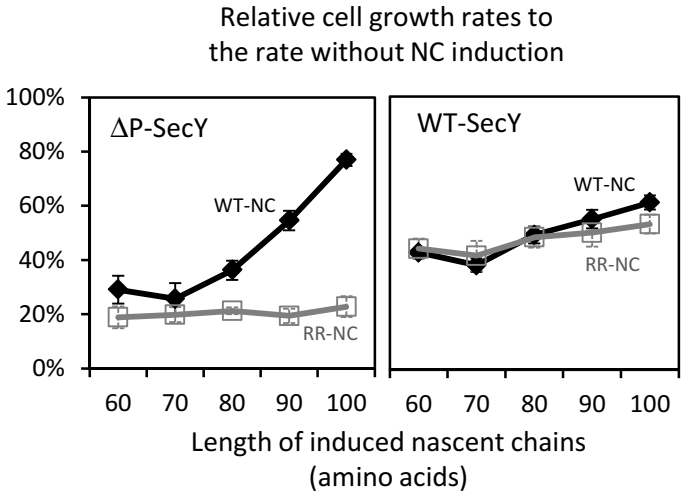
Supplementary Figure 6 | Dependence of nascent chain-SecY interaction on SRP and the hydrophobicity of the signal sequence.

a, NC100 with Cys at position 19 was expressed under the IPTG-inducible *tac* promoter (pTac-NC100) in WAM121 cells containing the chromosomal Ffh component of SRP under the arabinose (Ara) promoter. The cells were grown in the absence or presence of Ara and channel insertion of the nascent chain was probed by disulfide crosslinking to $SecY_{68C}$ after addition of the oxidant CuPh3. Where indicated, the samples were reduced with β-mercaptoethanol (β-ME) prior to non-reducing SDS-PAGE and immunoblotting with myc- or SecY- antibodies. SecY x NC~tRNA, SecY crosslink with NC100-tRNA. **b**, The cells used in **a** were analyzed by immunoblotting with Ffh antibodies. **c**, NC100 containing either wild-type (WT) or mutant DsbA signal sequences (shown in the lower panel), all with Cys at position 19, were expressed under the inducible arabinose promoter in EP51 cells expressing $SecY_{68C}$ (from pRSY). Where indicated, cell extracts were treated with 0.1 mM CuPh3 for 20 min at room temperature. The samples shown in lanes 4-6 were also treated with RNase A (lanes 7-9). The empty arrowhead indicates crosslinks of NC100 with Ffh.



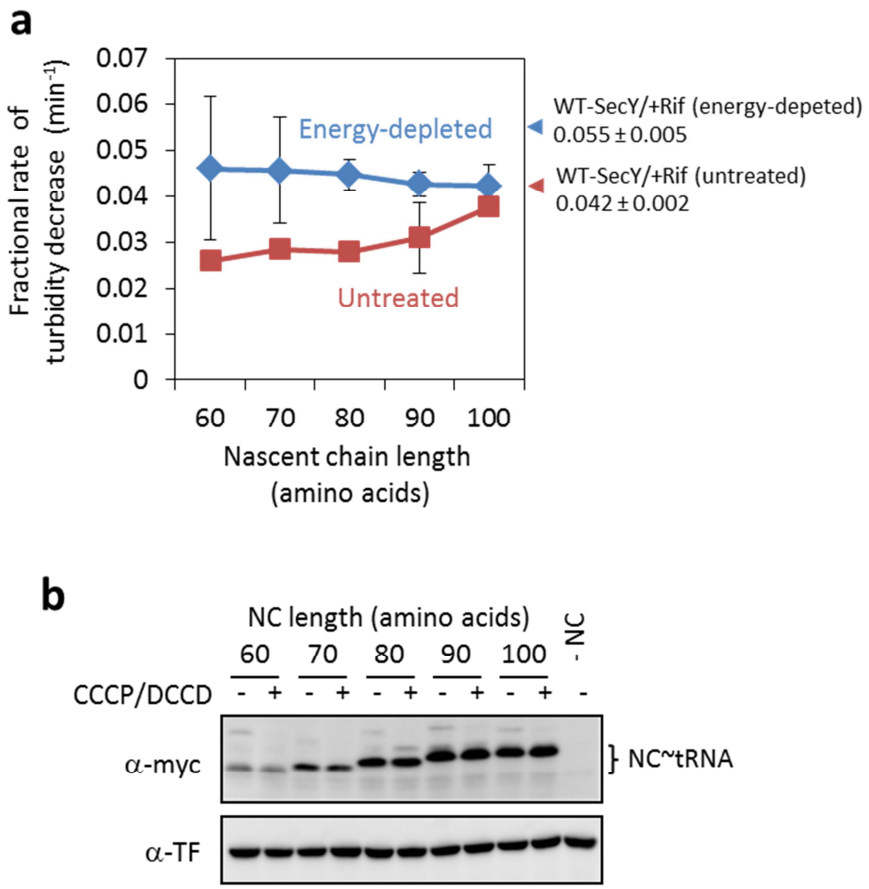
Supplementary Figure 7 | Blockage of BM permeation by nascent chain insertion into the open channel (ΔP).

Nascent chains of different lengths with either wild-type (WT-NC) or defective (RR-NC) signal sequence were expressed from the arabinose promoter in EP52 cells expressing the ΔP mutant of SecY from the constitutive endogenous promoter (pACYC-SecYEG; a GUG start-codon). As a control, NC100 synthesis was not induced (-NC). BM was added to the cells and biotinylated proteins detected by SDS-PAGE, followed by blotting with streptavidin-HRP conjugate. Relative biotinylation of p30 was quantified from three similar experiments and is shown in Figure 2e.



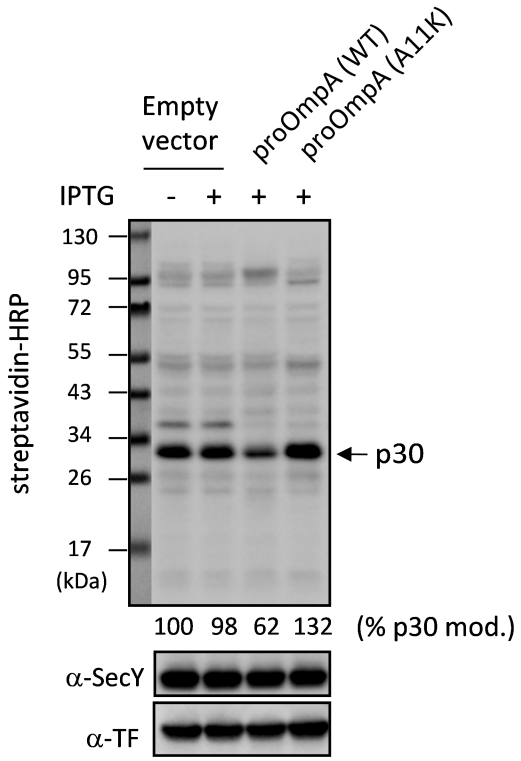
Supplementary Figure 8 | Growth rates of cells expressing nascent chains of various lengths.

Expression of nascent chains with different lengths was induced from the arabinose promoter in EP52 cells expressing either wild-type (WT) SecY or the ΔP mutant from the constitutive endogenous promoter (pACYC-SecYEG) and a GUG start-codon. The nascent chains contained either a wild-type (WT-NC) or defective (RR-NC) signal sequence. For each chain length, the growth rate was determined during the initial 1-hr arabinose induction [$(OD_{t=1h} - OD_{t=0})/ OD_{t=0}$] and normalized with the respect to the value obtained for uninduced cells (error bars, s.d.; *n*=3). OD, optical density at 600 nm.



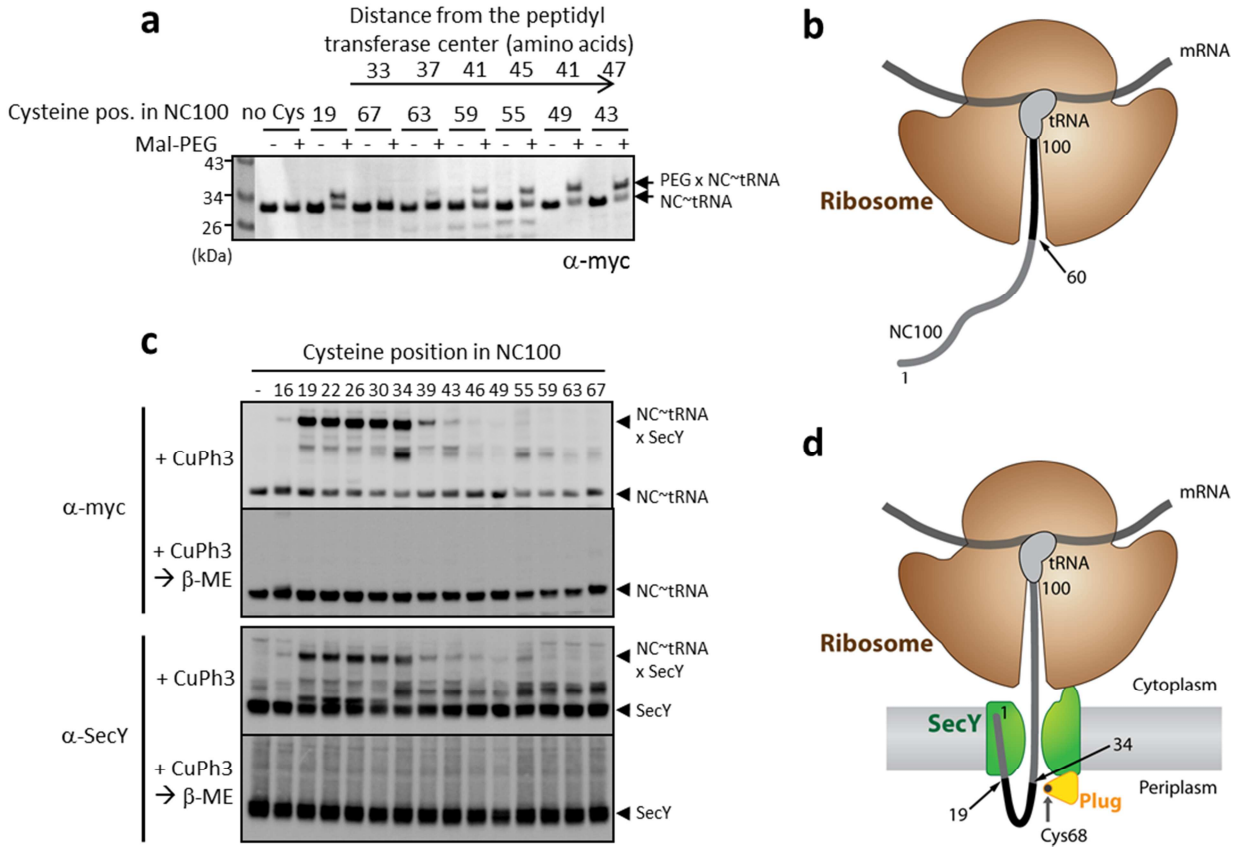
Supplementary Figure 9 | Cl^- ion permeability of the wild-type SecY channel containing different nascent chains.

a, EP53 cells expressing wild-type SecY together with nascent chains of different lengths (red squares) were converted into spheroplasts and diluted into an iso-osmotic solution of KCl containing valinomycin. The initial, linear rate of turbidity decease was measured (error bars, s.d.; *n*=3). To deplete energy (blue diamonds), the cells were treated prior to spheroplasting with 50 µM carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP; a proton-ionophore) and 50 µM *N,N'*-dicyclohexylcarbodiimide (DCCD, an F_0F_1 ATPase inhibitor) for 30 min at 37 °C. Spheroplasts were diluted into an iso-osmotic KCl solution containing 10 µM CCCP as well as valinomycin (error bars, s.d.; *n*=4). Note that the open channel (ΔP) without nascent chains gives a value of 1.06 (see Figure 2g). **b**, To verify the presence of nascent chains (NC) in cells after energy depletion, equal numbers of cells were collected before and after CCCP/DCCD-treatment and analyzed by immunoblotting with antibodies against the myc-tag (detects nascent chains) and trigger-factor (TF; loading control).



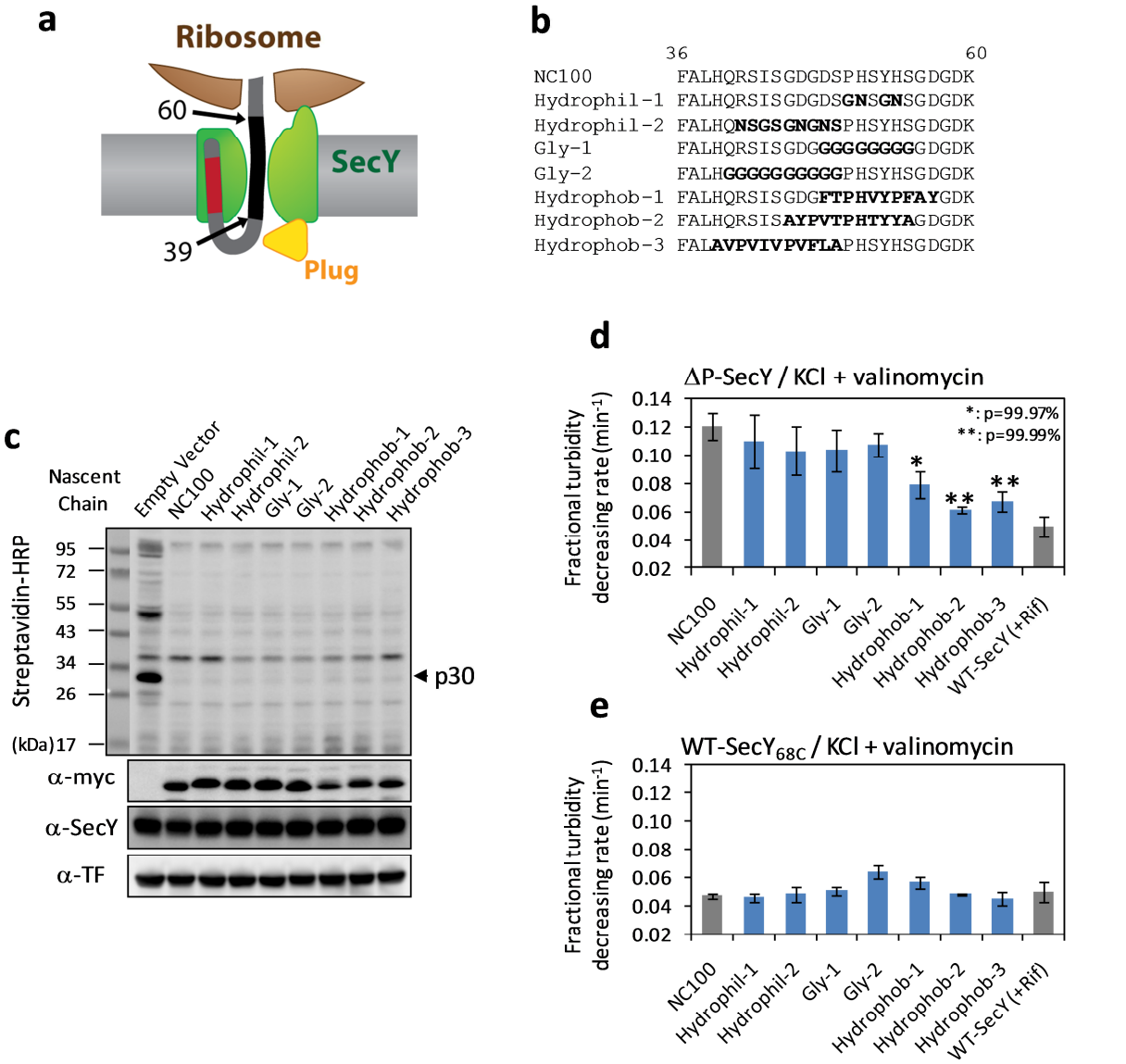
Supplementary Figure 10 | Permeability of the SecY channel during post-translational translocation.

The SecA-dependent post-translational substrate proOmpA was expressed for 1 hr at 37 °C under the *trc* promoter (pTrc99a-proOmpA) in the presence or absence of 1 mM isopropyl thiogalactopyranoside (IPTG) in EP52 cells expressing a plug-deletion (ΔP) mutant of SecY (from pACYC-SecYEG using a GUG start-codon). Controls were performed with a signal sequence mutant of proOmpA (A11K) or an empty vector. The cells were incubated for 15 min at room temperature with the modification reagent BM and the samples were analyzed by SDS-PAGE followed by blotting with streptavidin-HRP conjugate or SecY- and TF- antibodies. The modification of p30 was quantified by densitometry.



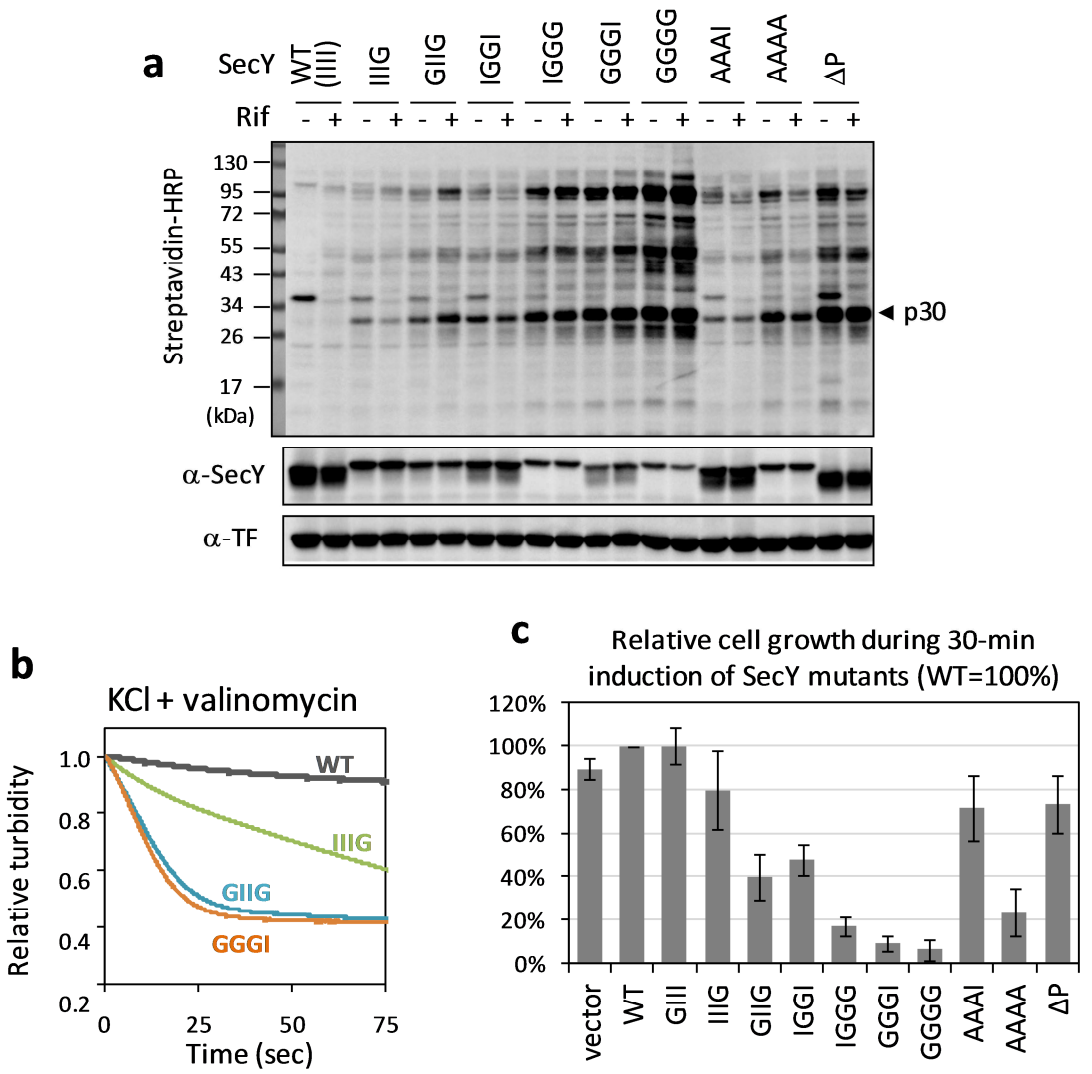
Supplementary Figure 11 | Mapping the residues in NC100 that are located in the ribosome exit tunnel or exposed on the periplasmic side of SecY.

a, NC100 chains with a cysteine at the indicated positions were expressed in EP51 cells for 2 hrs without SecY expression, and extracts were treated with maleimide-polyethylene glycol (Mal-PEG; 2.5 kDa). The samples were analyzed by SDS-PAGE and immunoblotting with myc antibodies. NC~tRNA and PEG x NC~tRNA, unmodified and modified peptidyl-tRNAs, respectively. **b**, Schematic for the results shown in **a**. The region of NC100 shown in black is protected from the Mal-PEG reagent by the ribosome exit tunnel. **c**, NC100 chains with a cysteine at the indicated positions were expressed for 2 hrs in EP51 cells expressing $SecY_{68C}$, (from pRSY), and disulfide bridge formation was induced with copper phenanthroline (CuPh3). The samples were analyzed by SDS-PAGE with or without reduction with β-mercaptoethanol (β-ME), followed by immunoblotting. NC~tRNA x SecY and NC~tRNA, crosslinked and non-crosslinked NC100-tRNA respectively. **d**, Schematic for the results shown in **c**. The region in black efficiently crosslinks with Cys68 of SecY.



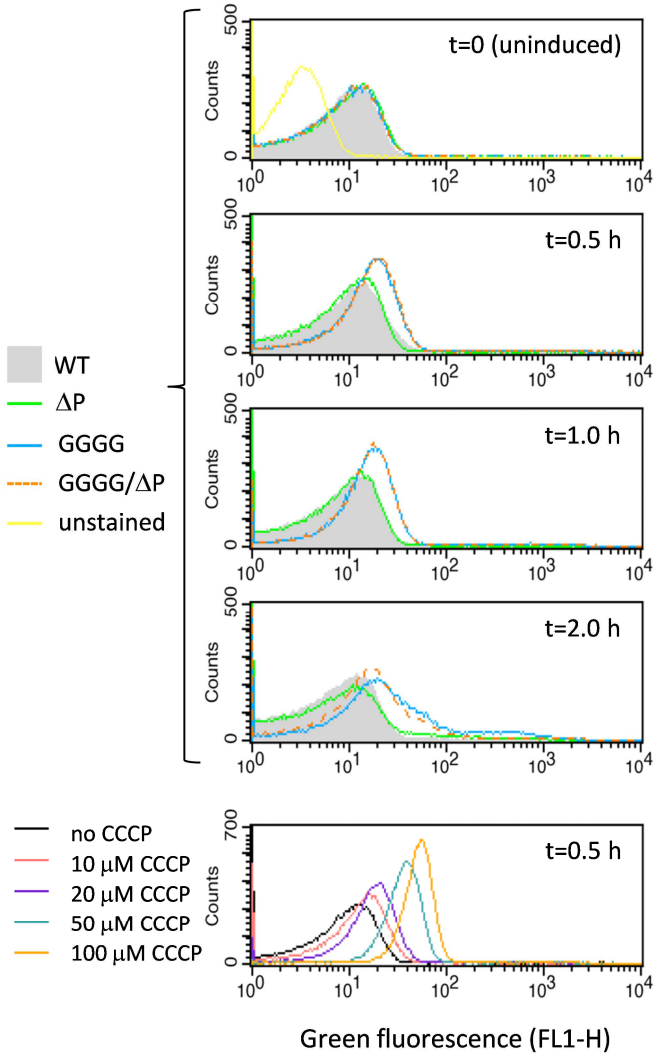
Supplementary Figure 12 | Effect of nascent chain sequence in the SecY channel on permeability for small molecules.

a, Scheme of NC100 in the SecY channel. **b**, Sequences of nascent chains placed inside the central pore of SecY. **c**, NC100 or its variants shown in **b** were synthesized in EP52 cells expressing ΔP SecY from the endogenous promoter (pACYC-SecYEG) and a GUG start-codon, then cells were incubated with the biotinylation reagent BM. Cell lysates were analyzed by SDS-PAGE followed by blotting with streptavidin-HRP conjugate, or SecY- and TF- antibodies. Control cells contained an empty vector. **d**, As in **c**, but spheroplasts were diluted into an iso-osmotic solution of KCl in the presence of valinomycin. The initial, linear rate of turbidity decrease was determined (error bars, s.d.; *n*=3). Stars indicate statistically significant changes relative to NC100, as determined by Students *t*-test. **e**, As in **d**, except that wild-type $SecY_{68C}$ was expressed.



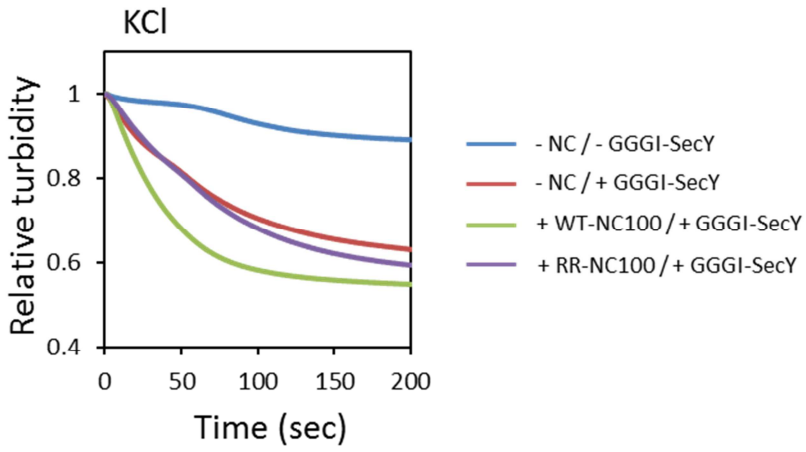
Supplementary Figure 13 | Effects of SecY pore mutations on BM or chloride permeability and on cell growth.

a, EP52 cells expressing wild-type (WT; IIII) SecY, the indicated pore mutants, or a plug-deletion (ΔP) mutant from the pTet-SecYEG vector were treated with the modification reagent BM. Where indicated, cells were treated with rifampicin (Rif) prior to BM addition. The samples were analyzed by SDS-PAGE followed by blotting with streptavidin-HRP or SecY- and TF- antibodies. **b**, Cells expressing wild-type SecY or the indicated pore mutants from pTet-SecYEG were converted into spheroplasts and diluted into an iso-osmotic solution of KCl containing valinomycin. Turbidity was monitored over time. **c**, As in **a**, The growth of cells used in **a** was measured during the initial 30-min induction of the channels. ($OD_{t=30m}$ - $OD_{t=0}$)/ $OD_{t=0}$ was calculated and normalized with respect to the value obtained for WT SecY-expressing cells (error bars, s.d.; *n*=3). OD, optical density at 600 nm.



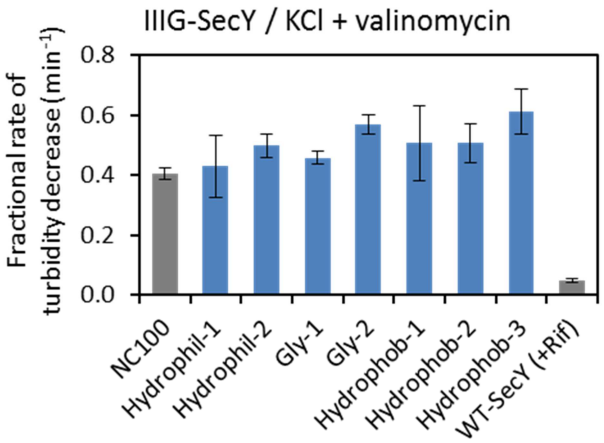
Supplementary Figure 14 | Membrane potential in cells expressing SecY mutants.

The expression of wild-type (WT) SecY, a plug-deletion (ΔP) mutant, a pore mutant with four glycine substitutions (GGGG), or a mutant containing both mutations (GGGG/ΔP) was induced with anhydrotetracycline in EP52 cells. At various times after induction, a voltage-sensitive dye (DIBAC4(3)) was added and the cells subjected to flow cytometry. For comparison, cells were treated with different concentrations of the H^+-ionophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) for 30 min. The yellow curve in the upper panel shows a WT sample without dye addition (unstained).



Supplementary Figure 15 | KCl permeation through SecY containing three glycine substitutions in the pore ring.

As in Figure 3b-d, NC100 with wild-type (WT) or defective (RR) signal sequence was expressed from the inducible arabinose promoter together with a SecY pore mutant, containing three glycine substitutions in the pore ring (GGGI), under the Tet promoter. Spheroplasts were diluted into an iso-osmotic KCl solution in the absence of valinomycin, and the turbidity change followed over time. “-NC” indicates that WT-NC100 was not induced.



Supplementary Figure 16 | Chloride permeation through a SecY pore mutant (IIIG) occupied by a nascent chain containing various sequences within the pore.

NC100 and its variants (see Supplementary Figure 12) were overproduced in EP53 cells constitutively expressing the pore mutant IIIG (from pACYC-SecYEG and a GUG start-codon). Spheroplasts were subjected to osmotic swelling and bursting in an iso-osmotic KCl solution containing valinomycin. The initial rates of turbidity decrease were measured (error bars, s.d.; *n*=3). As a control, cells were used that expressed wild-type (WT) SecY and were treated with rifampicin treatment.

SUPPLEMENTARY TABLES

Supplementary Table 1. Summary of strains used in this study

Name	Genotype/Description	Reference
BW25113	*F⁻ Δ(araD-araB)567 ΔlacZ4787(::rrnB-3) λ⁻ rph-1 Δ(rhaD-rhaB)568 hsdR514*	29
JW0936	BW25113 Δ*rmf::kan* (Keio collection)	28
JW0554	BW25113 Δ*ompT::kan* (Keio collection)	28
JW3898	BW25113 Δ*glpF::kan* (Keio collection)	28
Δrmf ΔompT (EP51)	BW25113 Δ*rmf ΔompT::kan* Construction: The kanamycin marker was removed from JW0936 by using pCP20 plasmid expressing Flp recombinase (ref. 29), and Δ*ompT::kan* was introduced by P1 transduction from JW0554.	This study
Δrmf ΔompT secY-CBP (EP52)	BW25113 Δ*rmf ΔompT::kan secY-CBP(Zeo^R)* Construction: the "CBP-RBS-Zeo" cassette was inserted immediately before the stop codon of the endogenous secY gene by Lamda Red recombination.	This study
Δrmf ΔompT secY-CBP ΔglpF (EP53)	BW25113 Δ*rmf ΔompT secY-CBP(Zeo^R)* Δ*glpF::Kan* Construction: The kanamycin marker was removed from the Δ*rmf* Δ*ompT secY-CBP* strain by using pCP20 plasmid, then Δ*glpF::kan* was introduced by P1 transduction from JW3898.	This study
WAM121	MC4100 *ara+ ffh::kan attB::(Ori$_{R6K}$ P$_{BAD}$-ffh tet)*	31

Supplementary Table 2. Summary of plasmids used in this study

Name	Description	Reference
pBAD myc-SecM	A plasmid for stalled nascent chain expression. Construction: the 10-residue myc-tag followed by the 17-residue SecM stalling sequence was inserted into the region between the *Pst*I and *EcoR*I sites of pBAD/His C (Invitrogen). There is an additional *Sac*I restriction site between the myc-tag and the SecM sequence.	This study
pBAD-NC100	A plasmid for NC100 nascent chain expression. Construction: A DNA sequence coding for the peptide sequence "*KKIWLALAGLVLAFSAS**C***AQYEDGSSGELERQHTFALHQRSISGDGDSPHSYHSGDGDSPHSYHSGDGDKLPEGVKMTKY" was synthesized and inserted between the *Nco*I and the *Pst*I sites of the pBAD myc-SecM vector. Because of the *Nco*I site, an additional alanine residue was incorporated right after the Met start-codon. The sequence corresponding to the DsbA signal peptide is shown as italicized. The original Ala19 residue was replaced by Cys (19C) (shown as a bold "C").	This study
pBAD-NC90	Expression plasmid for a shorter version of the NC100 nascent chain. Construction: the 10 amino acid residues immediately before the myc-tag (from the *Pst*I site to the N-terminus) were removed from pBAD-NC100 by PCR. Other truncated versions of NC100 (*i.e*. NC80, NC70, and NC60) were constructed in a similar way.	This study
pBAD-NC110	Expression plasmid for a longer version of the NC100 nascent chain. Construction: immediately before the myc-tag (the *Pst*I site) of pBAD-NC100, an additional 10-residue soluble segment (DGQSGSNGST) was inserted.	This study
pBAD-NC120	Expression plasmid for a longer version of the NC100 nascent chain. Construction: immediately before the myc-tag (the *Pst*I site) of pBAD-NC110, an additional 10-residue soluble segment (GNHGDSGSST) was inserted.	This study
pTac-NC100	A plasmid for expression of the NC100 nascent chain under an IPTG-inducible *tac* promoter. Construction: DNA encoding the NC100 nacent chain was amplified from pBAD-NC100 by PCR and inserted between the *Nde*I and *Hind*III sites of the pMAL-c2x vector (New England Biolabs)	This study

pBAD22-SecYEG	A plasmid for expression of the SecY complex. Polycistronic His-tagged SecE, SecY, and SecG coding sequences are under an arabinose-inducible promoter. SecY lacks cysteines (two endogenous Cys were replaced with Ser).	9
pTet	A vector with a tetracycline-inducible promoter (P_{LTet}). This vector contains the origin from pACYC184, a chloramphenicol resistance marker, TetR coding sequence (from Tn10), and the LTet promoter, and a multiple cloning site (MCS). The vector sequence and the map are available on request.	This study
pTet-SecYEG	A plasmid for expression of the SecY complex under a Tet inducible promoter. Construction: the polycistronic SecE/SecY/SecG coding region was PCR-amplified and inserted into pTet at its MCS site (KpnI/XhoI). SecY lacks cysteines.	
pTet-SecYEG(ΔP)	A plasmid for expression of the mutant SecY (ΔP) complex under a Tet inducible promoter. Construction: The residues 60-74 in the wild-type SecY of pTet-SecYEG was replaced with a short linker (GSGS) by Quikchange PCR.	This study, 9
pTet-SecYEG (GGGG) (and other SecY mutants)	A plasmid for expression of the four-glycine pore mutant SecY complex under a Tet inducible promoter. Construction: Four of six Ile pore residues in SecY (I86, I191, I278, and I408) were sequentially mutated to Gly by Quikchange PCR on pTet-SecYEG. Similarly, single or other multiple Gly (or Ala) pore mutants and *prl* mutants were constructed by Quikchange PCR.	This study
pACYC-SecYEG	A plasmid for expression of the SecY complex under the constitutive promoter of the *E. coli rplN* operon. Construction: the *rplN* promoter (P_{rplN}; the 200-bp upstream DNA fragment from the *E coli rplN* start-codon) was amplified by PCR from the *E. coli* chromosome and inserted into the *Sph*I-*Nco*I region of pBAD22-SecYEG replacing the AraC-P_{araBAD} fragment. The resulting P_{rplN}-SecE/SecY/SecG region was further amplified by PCR and inserted into the *XbaI-NruI* site of pACYC184. Where indicated, The residue Sec68 of SecY was mutated to Cys (denoted as "68C"), and/or the ATG start-codon to GTG. For the expression of mutant SecY complexes, corresponding mutations (*e.g*. plug-deletion) were introduced by PCR.	This study
pRARE2	Rare codon tRNA supplementing vector (Novagen)	
pHQ4	A plasmid for overexpression of SRP (Ffh, ffs) and the SRP receptor (FtsY) under their own native promoters.	32

pR2HQ4	A combined plasmid of pRARE2 and pHQ4. Construction: pRARE2 was linearized by cutting at the *Xba*I site and blunted with *Pfu* polymerase. The *Xba*I-*Sal*I restriction fragment containing the SRP and SR genes from pHQ4 was blunted and ligated into the linearized pRARE2 vector.	This study
pRSY	A plasmid for overexpression of SRP, the SRP receptor, and the SecY complex. Construction: pR2HQ4 was linearized by cutting at the *Nhe*I site. The P_{rplN}-SecE/SecY/SecG region was PCR-amplified from pACYC-SecYEG, digested at both ends with *Nhe*I, and ligated into the linearized pR2HQ4 plasmid.	This study
pRARE/SecYEG	A plasmid for overexpression of rare tRNA codons and SecYEG. Construction: similarly constructed as pRSY, except that pRARE2 was used instead of pR2HQ4.	This study
pBAD-NC100/SecYEG	A combined plasmid of pBAD-NC100 and pACYC-$SecY_{68C}$ EG, expressing the NC100 nascent chains under the *araBAD* promoter and the $SecY_{68C}$ complex under the *rplN* promoter. Construction: the P_{rplN}-SecEYG cassette was amplified by PCR from pACYC-$SecY_{68C}$ EG and inserted into the *Sph*I site of pBAD-NC100 by ligation. The ATG start-codon of SecY was changed to GUG by PCR.	This study
pTrc99a-proOmpA	A plasmid for expression of full-length proOmpA (lacks endogenous cysteines) under control of the IPTG-inducible *trc* promoter. Where indicated, the signal sequence of proOmpA contains the A11K mutation, which abolishes the export of proOmpA (ref. 4).	Lab collection

(Note) pTet, pACYC-SecYEG, pR2HQ4, pRSY, and pRARE/SecYEG contain a p15A origin of replication and a chloramphenicol acetyltransferase gene. The plasmids derived from pBAD, pTac, and pTrc99a contain a ColE1 origin of replication and a β-lactamase gene.

SUPPLEMENTARY DISCUSSION

In previous *in vitro* experiments, some permeation of Cl^- ions through the wild-type SecY channel was observed when inverted membrane vesicles contained a stalled SecA-dependent translocation intermediate[21] or when SecA-dependent translocation was initiated[14]. From the published data, we estimate that the detected Cl^- permeability was about two-fold higher than observed without translocation. In our *in vivo* experiments, the wild-type channel was essentially tight; no significant Cl^- permeation was observed, even when pump activity and membrane potential, which potentially could counteract Cl^- influx, were abolished by energy depletion. Possible explanations for the discrepancy are that the *in vitro* assay detects a very small Cl^- flux, much smaller than occurs with an open channel, or that SecA binding makes the SecY channel slightly more permeable, or that the SecY channel becomes more leaky after isolation of inverted vesicles. Proton leakage through the SecY channel was also reported[33]. Although these results could not be reproduced in similar, more recent studies[14], it is possible that H^+ ions move through SecDF, a channel-associated component that belongs to the RND transporter family.

The eukaryotic Sec61 channel has also been reported to have an intrinsic permeability for ions[5,6,34-37]. In contrast, the resting SecY channel is always tight (ref. 8 and this paper). Although it is possible that the Sec61 channel behaves differently from the SecY channel, more experiments are required to rule out that the Sec61 channel has an increased leakiness after isolation of microsomal membranes or of the Sec61 protein.

It is interesting that the open, resting SecY channel is closed for K^+, Na^+, and SO_4^{2-}ions, but permeable for Cl^- ions and for BM (ref. 14 and this paper). The size of the pore ring could exclude molecules if they exceed a certain size in their hydrated state. However, it is also possible that the hydrophobic nature of the pore ring contributes to discrimination; it could provide a barrier for most ions, but may be less effective in blocking Cl^- ions or hydrophilic, uncharged molecules, such as BM. Halides have been reported to move through lipid much more easily than Na^+ and K^+ ions[38,39], which might explain why they permeate more efficiently through the hydrophobic barrier in the SecY pore.

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