

Supplemental Table 1

List of the immobilized 13 residue long peptides used in the array.

SecY(PO3844) peptides

Y01	MAKQPLDFQSAK	Y21	IEMFNMFSGGALS	Y41	RYGTLVLAIFQSI
Y02	QPGLDFQSAKGGL	Y22	FNMFSGGALSRAS	Y42	TLVLAIFQSIGIA
Y03	LDFQSAKGGLGEL	Y23	FSGGALSRASIFA	Y43	LAIFQSIGIATGL
Y04	QSAKGGLGELKRR	Y24	GALSRASIFALGI	Y44	FQSIGIATGLPNM
Y05	KGGLGELKRRLLF	Y25	SRASIFALGIMPY	Y45	IGIATGLPNMPGM
Y06	LGELKRRLLFVIG	Y26	SIFALGIMPYISA	Y46	ATGLPNMPGMQGL
Y07	LKRRLLFVIGALI	Y27	ALGIMPYISASII	Y47	LPNMPGMQGLVIN
Y08	RLLFVIGALIVFR	Y28	IMPYISASIIQL	Y48	MPGMQGLVINPGF
Y09	FVIGALIVFRIGS	Y29	YISASIIQLLTV	Y49	MQGLVINPGFAFY
Y10	GALIVFRIGSFIP	Y30	ASIIQLLTVVHP	Y50	LVINPGFAFYFTA
Y11	IVFRIGSFIPIPG	Y31	IIQLLTVVHPTLA	Y51	NPFGAFYFTAVVS
Y12	RIGSFIPIPGIDA	Y32	LLTVVHPTLAEIK	Y52	FAFYFTAVVSLVT
Y13	SFIPIPGIDA AVL	Y33	VVHPTLAEIKKEG	Y53	YFTAVVSLVTGTM
Y14	PIPGIDA AVLAKL	Y34	PTLAEIKKEGESG	Y54	AVVSLVTGTMFLM
Y15	GIDA AVLAKLLEQ	Y35	AEIKKEGESGRK	Y55	SLVTGTMFLMWLG
Y16	AAVLAKLLEQORG	Y36	KKEGESGRRKISQ	Y56	TGTMFLMWLGEQI
Y17	LAKLLEQQRGTII	Y37	GESGRRKISQYTR	Y57	MFLMWLGEQITER
Y18	LLEQQRGTIEMF	Y38	GRRKISQYTRYGT	Y58	MWLGEQITERGIG
Y19	QQRGTIEMFNMF	Y39	KISQYTRYGTLVL	Y59	GEQITERGIGNGI
Y20	GTIEMFNMFSGG	Y40	QYTRYGTLVLAIF	Y60	ITERGIGNGISII

Y61	RGIGNGISIIIFA	Y81	QRRIVVNYAKRQQ	Y101	NWLTISLYLQPG
Y62	NGISIIIFAGIV	Y82	IVVNYAKRQQGRR	Y102	TTISLYLQPGQPL
Y63	ISIIIFAGIVAGL	Y83	NYAKRQQRRVYA	Y103	SLYLQPGQPLYVL
Y64	IIFAGIVAGLPPA	Y84	KRQQRRVYAAQS	Y104	LQPGQPLYVLLYA
Y65	AGIVAGLPPAIAH	Y85	QRRVYAAQSTHL	Y105	GQPLYVLLYASAI
Y66	VAGLPPAIAHTIE	Y86	RVYAAQSTHLPLK	Y106	LYVLLYASAI IFF
Y67	LPPAIAHTIEQAR	Y87	AAQSTHLPLKVN	Y107	LLYASAI IFFCFF
Y68	AIAHTIEQARQGD	Y88	STHLPLKVN MAGV	Y108	ASAI IFFCFFYTA
Y69	HTIEQARQGD LHF	Y89	LPLKVN MAGVIPA	Y109	I IFFCFFYTALVF
Y70	EQARQGD LHF LVL	Y90	KVN MAGVIPAIFA	Y110	FCFFYTALVFNPR
Y71	RQGD LHF LVL LLLV	Y91	MAGVIPAIFASSI	Y111	FYTALVFNPRETA
Y72	DLHF LVL LLLVAVL	Y92	VIPAIFASSIILF	Y112	ALVFNPRETADNL
Y73	FLV LLLVAVL VF A	Y93	AIFASSIILFPAT	Y113	FNPRETADNLKKS
Y74	LLL VAVL VF AVTF	Y94	ASSIILFPATIAS	Y114	RETADNLKKS GAF
Y75	VAVL VF AVTF FVV	Y95	IILFPATIASWFG	Y115	ADNLKKS GAFVPG
Y76	LVFAVTF FV FVE	Y96	FPATIASWFGG GT	Y116	LKKS GAFVPGIRP
Y77	AVTF FV FVERGQ	Y97	TIASWFGG GTGWN	Y117	SGAFVPGIRPGEQ
Y78	FFV FVERGQ RRI	Y98	SWFGG GTGWNWLT	Y118	FVPGIRPGEQTAK
Y79	VFVERGQ RRI VVN	Y99	GGGTGWNWLT TIS	Y119	GIRPGEQTAKYID
Y80	ERGQ RRI VVNYAK	Y100	TGWNWLT TISLYL	Y120	PGEQTAKYIDKVM

Y121	QTAKYIDKVMTRL	Y131	MRDAMKVPFYFGG	Y141	QTLMMSSQYESAL
Y122	KYIDKVMTRLTLV	Y132	AMKVPFYFGGTSL	Y142	MMSSQYESALKKA
Y123	DKVMTRLTLVGAL	Y133	VPFYFGGTSL LIV	Y143	SQYESALKKANLK
Y124	MTRLTLVGALYIT	Y134	YFGGTSL LIVVVV	Y144	ESALKKANLKGYG
Y125	LTLVGALYITFIC	Y135	GTSLLIVVVVIMD	Y145	SALKKANLKGYGR
Y126	VGALYITFICLIP	Y136	LLIVVVVIMDFMA		
Y127	LYITFICLIPEFM	Y137	VVVVIMDFMAQVQ		
Y128	TFICLIPEFMRDA	Y138	VIMDFMAQVQ TLM		
Y129	CLIEPEFMRDAMKV	Y139	DFMAQVQ TLMSS		
Y130	PEFMRDAMKVPFY	Y140	AQVQ TLMSSQYE		

SecE (P16920) peptides

E01	MSANTEAQSGSRG	E21	LTTKGKATVAFAR
E02	NTEAQSGSRGLEA	E22	KGKATVAFAREAR
E03	AQSGSRGLEAMKW	E23	ATVAFAREARTEV
E04	SGRGLEAMKWVVV	E24	AFAREARTEVRKV
E05	GLEAMKWVVVVAL	E25	REARTEVRKVIWP
E06	AMKWVVVVALLLV	E26	RTEVRKVIWPTRO
E07	WVVVVALLLVVAIV	E27	VRKVIWPTROETL
E08	VVALLLVVAIVGNY	E28	VIWPTROETLHTT
E09	LLLVAIVGNYLYR	E29	PTRQETLHTTLIV
E10	VAIVGNYLYRDIM	E30	QETLHTTLIVAAV
E11	VGNLYRDIMLPL	E31	LHTTLIVAAVTAV
E12	YLYRDIMLPLRAL	E32	TLIVAAVTAVMSL
E13	RDIMLPLRALAVV	E33	VAAVTAVMSLILW
E14	MLPLRALAVVILI	E34	VTAVMSLILWGLD
E15	LRALAVVILIAAA	E35	VMSLILWGLDGIL
E16	LAVVILIAAAGGV	E36	LILWGLDGILVRL
E17	VILIAAAGGVALL	E37	WGLDGILVRLVSF
E18	IAAAGGVALLTTK	E38	DGILVRLVSFITG
E19	AGGVALLTTKGKA	E39	LVRLVSFITGLRF
E20	VALLTTKGKATVA		

SecG (P33582) peptides

G01	MYEALLVVFLIVA	G21	TLFFIISLVLGNI
G02	ALLVVFLIVAIGL	G22	FIISLVLGNINSN
G03	VVFLIVAIGLVGL	G23	SLVLGNINSNKTN
G04	LIVAIGLVGLIML	G24	LGNINSNKTNKGS
G05	AIGLVGLIMLQQG	G25	INSNKTNGSEWE
G06	LVGLIMLQQGKGA	G26	NKTNGSEWENLS
G07	LIMLQQGKGADMG	G27	NKGSEWENLSAPA
G08	LQQGKGADMGASF	G28	SEWENLSAPAKTE
G09	GKGADMGASFGAG	G29	ENLSAPAKTEQTQ
G10	ADMGASFGAGASA	G30	SAPAKTEQTQPAA
G11	GASFGASATLFF	G31	AKTEQTQPAAPAK
G12	FGAGASATLFGSS	G32	QTQPAAPAKPTS
G13	GASATLFGSSGSG	G33	QPAAPAKPTSDIP
G14	ATLFGSSGSGNFM	G34	PAAPAKPTSDIPN
G15	FGSSGSGNFMTRM		
G16	SGSGNFMTRMTAL		
G17	GNFMTRMTALLAT		
G18	MTRMTALLATLFF		
G19	MTALLATLFFIIS		
G20	LLATLFFIISLVL		

E06			AMKVVVVVALLLV	1.3	
E07			WVVVVALLLVAIV	0.38	20 30 40 50
E08			VVALLLVAIVGNY	0.52	
E09	LLLVVAIVGNYLYR	0.29	LLLVVAIVGNYLYR	0.48	MBS-4 AMKVVVVVALLLVAIVGNYLYRDIPLRALAVVILIAAAGGV
E10	VAVGNYLYRDI	0.42	VAVGNYLYRDI	0.81	MBS-37 AMKVVVVVALLLVAIVGNYLYRDIPLRALAVVILIAAAGGV
E11	VGNYLYRDIPL	2.26	VGNYLYRDIPL	1.27	Cons-B
E12	LYRDIPLRAL	1.41	LYRDIPLRAL	1.1	Cons-E+A
...					
E14			MLPLRALAVVILI	0.91	
E15			LRALAVVILIAAA	0.35	
E16			LAVVILIAAAGGV	1.47	
E19	AGGVALLTTKGA	0.45			60 70 80 90
E20	VALLTTK GKATVA	0.7			MBS-4 VAL LTTK GKATVAFAREARTEVRKVIWPTRQETL
E21	LTTK GKATVAFAR	1.12			MBS-37 VALLTTK GKATVAFAREARTEVRKVIWPTRQETL
E22	K GKATVAFAR EAR	0.62			Cons-B
E23	ATVAFAREARTEV	0.26			Cons-E+A
E24	AFAREARTEVRKV	0.78			
E25	REARTEVRKVIWP	0.49			SS HHHHHHHHHHHHHHHHHHHHH-----HHHH.....
E26	RTEVRKVIWPTRQ	0.27			C1 TM2
E27	VRKVIWPTRQETL	0.68			3D-BS VAL LTTK GKATVAFAREARTEVRKVIWPTRQETL
					$\gamma 4$
E35			VMSLILWGLDGIL	0.7	110 120
E36			LILWGLDGILVRL	2.25	
E37			WGLDGILVRLVSF	1.61	MBS-4 VMSLILWGLDGILVRLVSFITGLRF
E38			DGILVRLVSFITG	2.08	MBS-37 VMSLILWGLDGILVRLVSFITGLRF
E39			LVRLVSFITGLRF	0.55	Cons-B
					Cons-E+A
					SS HHHHHHHHHHHHHHHHHHHHH-----
					TM2 P1
					3D-BS VMSLILWGLDGILVRLVSFITGLRF
					$\beta 1$
SecG					
G01	MYEALLVFLIVA	0.47	MYEALLVFLIVA	1	1 10
G02	ALLVFLIVAIGL	0.05	ALLVFLIVAIGL	0.43	
G03			VVFLIVAIGLVGL	0.43	MBS-4 MYEALLVFLIVAIGLVGL
					MBS-37 MYEALLVFLIVAIGLVGL

G18			MTRMTALLATLFF	0.47	50	60	70	90
G19			MTALLATLFFIIS	1.17				
G20			LLATLFFIISLVL	0.88	MBS-4	NFMTRMTALLATLFFIISLVL	LGNI	NSNKTKNGS
G21			TLFFIISLVLGNI	0.91	MBS-37	NFMTRMTALLATLFFIISLVL	GNINSN	KTKNGS
G22			FIISLVLGNI	1.9	Cons-B			
G23	SLVLGNINSNKTN	0.17			Cons-E+A			
G24	LGNI	2.54						

SS	-----	HHHHHHHHHHHHHHHHHHHHHHHHHHHH	-----
	C1	TM2	P1
3D-BS-4	NFMTRMTALLATLFFIISLVL	LGNI	NSNKTKNGS
			β3
3D-BS-37	NFMTRMTALLATLFFIISLVL	GNINSN	KTKNGS
			β2

Quantitation of binding, minimal binding sequence (MBS) determination and sequence conservation [within the Bacteria (Cons-B) and within Eukaryotes and Archaea (Cons-E+A)]. "Binding Strength" was determined as follows: The density of all spots on the array was scored following scanning densitometry and visual inspection. Values from 2-4 repeat experiments were averaged. Delineation of minimal binding sequences was initiated with the peptide giving the highest score, then proceeding with the flanking peptides. Highlighted residues (4°C, green; 37°C, yellow) are the ones that appear to contribute to binding. Only signals that include surface exposed residues from peptides with a binding strength score above 0.5 (4°C) and 1.25 (37°C) were included in the determination of the MBS. At 37°C some of the peptides with extensive hydrophobic character were disregarded.

Colour code for residue conservation: Red= 100% ; Light blue= >70% identical or of similar property. @= acidic; += basic; s=small (A,G); o= T,S, h=hydrophobic (ILVMA); b= bulky (F,Y,W). All residue numbering is for the corresponding *E.coli* protein.

The consensus sequence for was derived from multiple alignment of sequences from the following organisms (provided with their exspasy codes):

Bacteria: [Aquifex aeolicus O66491; Chlamydia pneumoniae Q9Z7S5; Thermotoga maritima Q9X1I9; Nitrosospira multiformis Q2YAX7; Cytophaga hutchinsonii Q11QD2; Desulfotobacterium hafniense Q250L2; Shigella boydii Q31VX6; Yersinia enterocolitica A1JS07; Aeromonas salmonicida A4SSY6; Shewanella oneidensis Q8EK50; Actinobacillus pleuropneumoniae A3N377; Vibrio cholerae P78283; Acidovorax avenae A1TJT7; Xanthomonas axonopodis pv. citri Q8PNQ8; Bordetella bronchiseptica Q7WRA3; Legionella pneumophila Q5WZJ2; Neisseria meningitidis A1INX0; Bradyrhizobium japonicum Q89JA4; Syntrophobacter fumaroxidans AOLILO; Agrobacterium tumefaciens Q8UE37; Acidiphilium cryptum A5FZU5]

Archaea: [Aeropyrum pernix Q9YDD0; Methanobrevibacter smithii A5UL65; Hyperthermus butylicus A2BME2; Methanosphaera stadtmanae Q2NFY0; - Methanosphaera stadtmanae Q2NFY0; Archaeoglobus fulgidus O28377; Ignicoccus hospitalis A8ABS3; - Methanococcus jannaschii Q60175; Sulfolobus acidocaldarius P49978; Sulfolobus acidocaldarius P49978; Methanopyrus kandleri Q8TZA9; Halorubrum lacusprofundi A7D110; Pyrococcus furiosus Q8U019; Methanosarcina barkeri Q46GB8; Metallosphaera sedula A4YCY9; Halobacterium salinarium Q9HPB1; Cenarchaeum symbiosum A0RUE4; Natronomonas pharaonis Q3IMW5; Natronomonas pharaonis Q3IMW5; Thermofilum pendens A1RWR3; Picophilus torridus Q6L1A4.

Eukaryotes: [Rhodomonas salina A6MW18; Porphyra yezoensis Q1XDJ1; Ostreococcus lucimarinus A4RW47; Odontella sinensis P49461; Arabidopsis thaliana Q9SIQ4; Schizosaccharomyces pombe P79088; Chaetomium globosum Q2GWS9; Neurospora crassa. Q870W0; Aspergillus clavatus. A1CAP3; Leishmania major Q4QGX4; Caenorhabditis elegans O18239; Homo sapiens Q9H9S3; Mus musculus Q9CYJ6; Canis familiaris P38377; Anopheles gambiae Q7PVU5; Drosophila pseudoobscura Q29PD8]

SS=Secondary structure. Information was extrapolated from the *M.jannaschi* structure after pairwise alignment of the two sequences. This assignment is different in some parts of the sequence from the derived 3-D models. H= α -helix, S= β -strand, dash=loop. The transmembrane regions (TM) of SecYEG and the periplasmic (P) and cytoplasmic (C) regions are indicated (as in Fig. 2A). *, unresolved structure.

3D-BS= three dimensional binding sites. Minimal binding sequences were mapped on the three dimensional structure model of ecSecYEG derived from structural modelling using the *M.jannaschii* crystal structure (Fig. 1A). SecA binding residues fall in 5 discrete clusters of binding sites named α - ϵ (Fig. 2A-F and H-J) and coloured as follows: α = light red, β = light orange, γ = light yellow, δ = light green, ϵ = light purple. Groups of binding residues within the same binding site are numbered (α 1, α 2 etc).

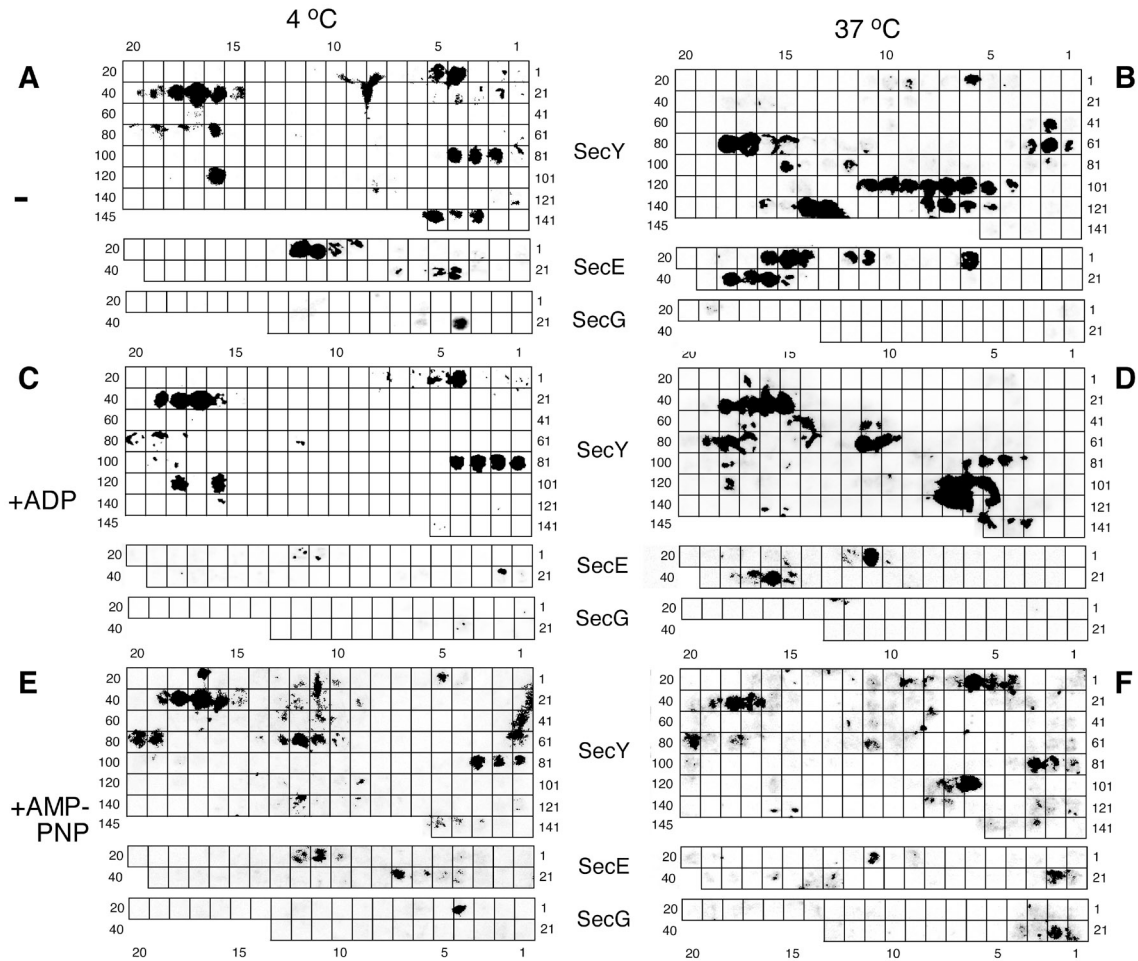
Supplemental Table 3

List of SecY mutants generated in this study and which express SecY to wild-type levels in their membranes. Values of *in vivo* complementation of a chromosomal *secYcs* mutant is also shown. In the same complementation assay the wild type SecY expressed from plasmid pET610 allows growth equivalent to "++++" while the vector without an insert does not allow any growth "-"). Values in parentheses indicate faint growth at the corresponding dilution. All substitutions are to alanyl residues. NT=not tested. Colouring of the rows is as in Fig. 2 and indicates different SecA binding sites of SecYEG.

	Binding site	Mutant	Mutated residues	In vivo complementation	Class	Membrane-inserted SecY in <i>secYcs</i>	Membrane-inserted SecY in BL31
1	ϵ_1	M1	²⁰ KRR ²³	++++	WT	+	NT
2	ϵ_1	M2	²² RLLF ²⁵	++	III	+	NT
3	α_1	M17	¹⁰⁹ GE ¹¹⁰	+++	III	NT	+
4	α_1	M4	¹¹² GRRK ¹¹⁵	+	II	+	+
5	α_1	M18	¹¹⁸ Q	+++(+)	IV	NT	+
6	α_1	M19	¹¹⁹ Y	+++	III	NT	+
7	α_1	M20	¹²⁰ T	+++	III	NT	+
8	α_1	M21	¹²¹ R	+	I	NT	+
9	γ_2	M36	²³⁸ ER ²³⁹	++(+)	III	NT	NT
10	γ_2	M7	²⁴⁰ QRR ²⁴²	+++	III	+	+
11	δ_1	M8	²⁴⁸ YAKR ²⁵¹	+++(+)	IV	+	NT
12	δ_1	M9	²⁵⁴ RRVY ²⁵⁷	+++	III	+	NT
13	δ_2	M11	³⁴⁷ KKS ³⁴⁹	+(+)	II	+	NT
14	δ_2	M22	³⁵⁶ IR ³⁵⁷	+	I	NT	+
15	δ_2	M23	³⁵⁸ PG ³⁵⁹	+	I	NT	+
16	δ_3	M13	³⁶⁴ KYID ³⁶⁷	+(+)	II	+	NT
17	δ_3	M24	³⁷¹ RL ³⁷²	+	I	NT	NT
18	δ_3	M25	³⁷³ T	+++	III	NT	NT
19	ϵ_2	M15	⁴²⁸ QYE ⁴³⁰	-	I	+	+
20	ϵ_2	M10	⁴³³ LKK ⁴³⁵	++	II	+	NT
21	ϵ_2	M16	⁴³⁷ NLK ⁴³⁹	++++	IV	+	NT

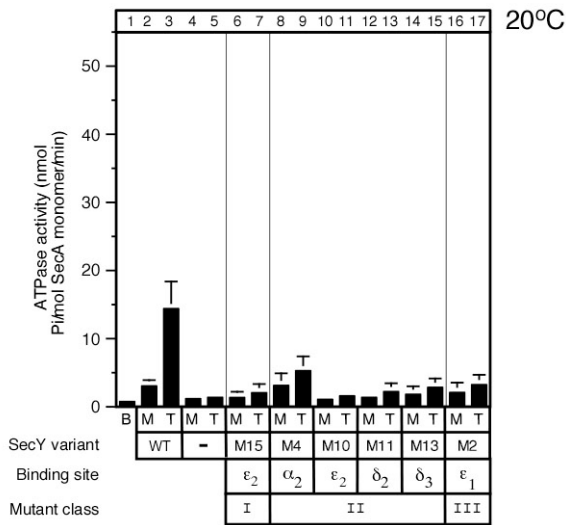
Supplemental Figure 1

SecA binding to SecYEG peptide arrays as a function of temperature and nucleotide. Binding of SecA to the array at the indicated conditions was carried as described in Fig. 1B.



Supplemental Fig. 3

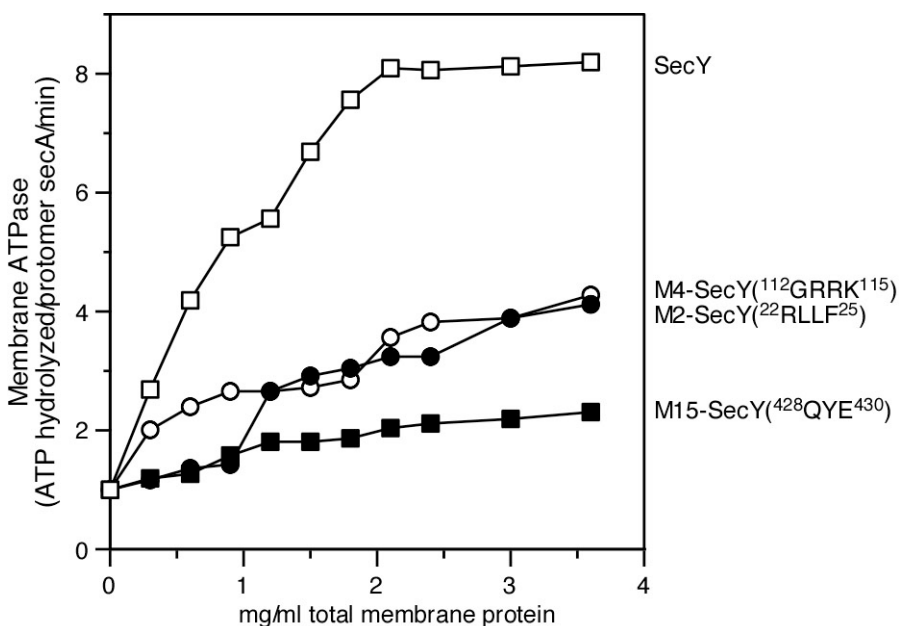
A. *In vitro* translocation ATPase assays at 20°C.



K_{cat} values of basal, membrane (urea-treated IMVs; 17

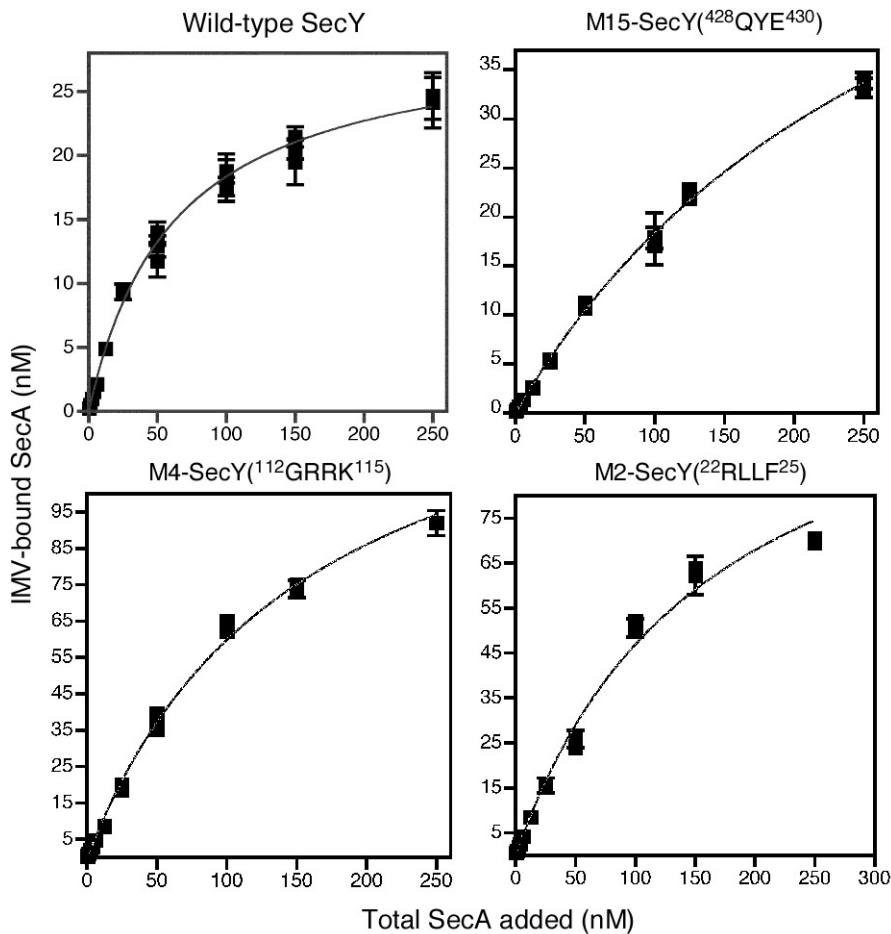
μg protein/ml) and translocation (IMVs plus 60 $\mu\text{g}/\text{ml}$ proOmpA) ATPase activities of SecY and of its indicated derivatives were determined at 20°C. To exclude background values from the small amounts of the chromosomally-encoded SecY, IMVs prepared from strain AF569 carrying the vector alone were also prepared. Values for these IMVs were determined in the same experiment and were subtracted from the corresponding values of the IMVs harbouring mutant SecYs.

B. Dependence of "membrane ATPase" on concentration of SecYEG-harboring membrane vesicles.



Supplemental Fig. 4

Quantitation of SecA binding to SecYEG.



Binding of [³⁵S]-labelled SecA proteins to inner membrane vesicles (IMVs) was performed as described elsewhere (Hartl et al, 1990; Vrontou et al, 2004; Karamanou et al, 2007). Briefly, urea-treated IMVs (64 μg/ml) were mixed with a range of ³⁵S-labelled SecA concentrations (0.5-2000 nM; buffer B; 1mg/ml BSA; 15min; 4°C). Reactions were overlaid on equal volume of buffer B, 0.2 M sucrose, 1mg/ml BSA, in centrifuge tubes preadsorbed with BSA and sedimented (320,000 x g; 30 min; 4°C; Beckman TLX120 ultracentrifuge). Pellets, rinsed (two times; 100ul of buffer B) and resuspended by sonication, were spotted on nitrocellulose membranes in a vacuum manifold (Bio-Rad). Bound radioactivity was quantitated by phosphorimaging. Data were fitted to hyperbolae using nonlinear regression in Prism (GraphPad).

Supplemental Materials and Methods

Our mutagenesis strategy was as follows: a. To avoid mutants with unwanted membrane insertion and assembly defects we focused solely on cytoplasmic or membrane peripheral regions (Fig. 4A). b. Since SecY forms the highest number of SecA contacts (Fig. 2) and is essential for the secretion channel was mutagenized. c. To enhance the mutagenic phenotype we resorted to generating multiple (3-5) alanine substitutions in consecutive residues. This was for two reasons: a. previous studies have suggested that in many cases single residue substitutions in SecY (Mori and Ito, 2001; van der Sluis et al., 2002; Tam et al., 2005) or other membrane transporters (Frillingos et al., 1998) do not yield measurable phenotypes. b. the binding sites we identified here are of low affinity. d. Candidate target residues were screened against a multiple alignment of selected SecY proteins from Bacteria and one from the other two Domains. In most cases residues conserved in Bacteria but not in the other Domains were selected for mutagenesis.

Construction of M01 (SecY²⁰KRR²³/AAA): SecY²⁰KRR²³/AAA was constructed by “megaprimer” PCR using pET610 (van der Does et al., 1998) as template and X331 (5’ CACACAGGAAACAGACCATGCATCACCATCAC3’) as forward primer and X347 (5’ CAGCGCACCGATAACAAACAGCAGT**GCGGCCG**CAGCTCGCCTAAGCCACCTTTG3’) as reverse mutagenic primer. The 0.15kb PCR product was used in a second step PCR with X339 (5’ GTATTCGCACCCATGGTCTGTTTCC3’) as reverse primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB586.

Construction of M02 (SecY²²RLLF²⁵/AAAA): SecY²²RLLF²⁵/AAAA was constructed by “megaprimer” PCR using pET610 as template and X331 (5’ CACACAGGAAACAGACCATGCATCACCATCAC3’) as forward primer and X348 (5’

CACAATCAGCGCACCGATAAC**AGCGGCCGCTGCGCGTTTCAGCTCGCCTAAGCC** 3') as reverse mutagenic primer. The 0.15kb PCR product was used in a second step PCR with X339 (5' GTATTCGCACCCATGGTCTGTTTCC3') as reverse primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB587.

Construction of M17 SecY¹⁰⁹GE¹¹⁰/AA: SecY¹⁰⁹GE¹¹⁰/AA was constructed was constructed USING Quick-Change PCR site-directed Mutagenesis (Stratagene) using *PfuUltraTM* High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X456(5-GCA GAA ATT AAG AAA GAA GCG GCG TCT GGT CGT CGT AAG ATC-3) was the forward mutagenic primer and X457 (5-GAT CTT ACG ACG ACC AGA CGC CGC TTC TTT CTT AAT TTC TGC-5) was the reverse. This resulted in construct pIMBB843.

Construction of M04 (SecY¹¹³RRK¹¹⁵/AAA): SecY¹¹³RRK¹¹⁵/AAA was constructed by “megaprimer” PCR using pET610 as template and X331 (5' CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer and X330 (5' CGTAGCGGGTGTACTGGCTGAT**CGCTGCAGC**ACCAGACTCCCCTTCTTTCTTAATTTCC3') as reverse mutagenic primer. The 0.4kb PCR product was used in a second step PCR with X339 (5' GTATTCGCACCCATGGTCTGTTTCC3') as reverse primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB579.

Construction of M18 (SecY¹¹⁸Q/A): SecY¹¹⁸Q/A was constructed using Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltraTM* High Fidelity DNA

polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X458 (5'-CGT CGT AAG ATC AGC GCG TAC ACC CGC TAC GGT-3') was the forward mutagenic primer and X459 (5'-ACC GTA GCG GGT GTA CGC GCT GAT CTT ACG ACG-3') was the reverse. This resulted in construct pIMBB861.

Construction of M19 (SecY¹¹⁹Y/A): SecY¹¹⁹Y/A was constructed using Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltra*TM High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X460 (5'-CGT AAG ATC AGC CAG GCC ACC CGC TAC GGT ACT-3') was the forward mutagenic primer and X461 (5'-AGT ACC GTA GCG GGT GGC CTG GCT GAT CTT ACG-3') was the reverse. This resulted in construct pIMBB862.

Construction of M20 (SecY¹²⁰T/A): SecY¹²⁰T/A was constructed using Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltra*TM High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X462(5- AAG ATC AGC CAG TAC GCC CGC TAC GGT ACT CTG-3) was the forward mutagenic primer and X463 (5-CAG AGT ACC GTA GCG GGC GTA CTG GCT GAT CTT-3) was the reverse. This resulted in construct pIMBB863.

Construction of M21 (SecY¹²¹R/A): SecY¹²¹R/A was constructed using Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltra*TM High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X464(5- ATC AGC CAG TAC ACC GCC TAC GGT ACT CTG GTG-) was the forward mutagenic primer and X465 (5- CAC CAG AGT ACC GTA GGC GGT GTA CTG GCT GAT-3) was the reverse. This resulted in construct pIMBB843.

Construction of M36 (SecY²³⁸ER/AA): SecY²³⁸ER/AA was constructed using Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltra*TM High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X498(5- TTC TTT GTT GTA TTT GTT GCG GCT GGT CAA CGC CGC ATT GTG GTA 35- TTC TTT GTT GTA TTT GTT GCG GCT GGT CAA CGC CGC ATT GTG GTA 3) was the

forward mutagenic primer and X499 (: 5-TAC CAC AAT GCG GCG TTG ACC AGC CGC AAC AAA TAC AAC AAA GAA -3) was the reverse. This resulted in construct pIMBB843.

Construction of M07 (SecY²⁴⁰QRR²⁴²/AAA): SecY²⁴⁰QRR²⁴²/AAA was constructed by

“megaprimer” PCR using pET610 as template and X353 (5’

GTTGTATTTGTTGAGCGTGGT**GCGGCCGCC**ATTGTGGTAAACTACGCGAAACG 3’) as

forward mutagenic primer and X339 (5’ GTATTCGCACCCATGGTCTGTTTCC3’) as reverse

primer. The 0.6kb PCR product was used in a second step PCR with X331 (5’

CACACAGGAAACAGACCATGCATCACCATCAC3’) as forward primer. The final 1.4kb PCR

product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in

pIMBB590.

Construction of M08 (SecY²⁴⁸YAKR²⁵¹/AAAA): SecY²⁴⁸YAKR²⁵¹/AAAA was constructed by

“megaprimer” PCR using pET610 as template and X372 (5’

CAACGCCGCATTGTGGTAAAC**GCCGCGCGCT**CAGCAAGGTCGTCTGTCTATG 3’) as

forward mutagenic primer and X339 (5’ GTATTCGCACCCATGGTCTGTTTCC3’) as reverse

primer. The 0.6kb PCR product was used in a second step PCR with X331 (5’

CACACAGGAAACAGACCATGCATCACCATCAC3’) as forward primer. The final 1.4kb PCR

product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in

pIMBB591.

Construction of M09 (SecY²⁵⁴RRVY²⁵⁷/AAAA): SecY²⁵⁴RRVY²⁵⁷/AAAA was constructed by

“megaprimer” PCR using pET610 as template and X357 (5’

CTACGCGAAACGTCAGCAAGGT**GCTGCGCGCT**GCTGCACAGAGCACACATTTAC3’) as

forward mutagenic primer and X339 (5’ GTATTCGCACCCATGGTCTGTTTCC3’) as reverse

primer. The 0.6kb PCR product was used in a second step PCR with X331 (5’

CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB600.

Construction of M10 (SecY⁴³³LKK⁴³⁵/AAA): SecY³³³LKK³³⁵/AAA was constructed by "megaprimer" PCR using pET610 as template and X362 (5'

GTCCAGTCAGTATGAGTCTGCAG**CGGCGCGG**CGAACCTGAAAGGCTACGGCC3') as forward mutagenic primer and X355 (5' GTTAAGAGCGCGACACCACCCGCTGC 3') as reverse primer. The 0.25kb PCR product was used in a second step PCR with X331 (5'

CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB604.

Construction of M11 (SecY³⁴⁷KKS³⁴⁹/AAA): SecY³⁴⁷KKS³⁴⁹/AAA was constructed by "megaprimer" PCR using pET610 as template and X358 (5'

GCGTGAAACAGCAGATAACCTG**GCGGCGCG**CGGTGCATTTGTACCAGGAATTC3') as forward mutagenic primer and X339 (5' GTATTCGCACCCATGGTCTGTTTCC3') as reverse primer. The 0.35kb PCR product was used in a second step PCR with X331 (5'

CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB592.

Construction of M22 (SecY³⁵⁶IR³⁵⁷/AA) was constructed using Quick-Change PCR site-directed Mutagenesis (Stratagene) using *PfuUltra*TM High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X466 (5'-GGT GCA TTT GTA CCA GGA GCT GCA CCG GGA GAG CAA ACG GCG-3') was the forward mutagenic

primer and X467 (5-CGC CGT TTG CTC TCC CGG TGC AGC TCC TGG TAC AAA TGC ACC-3) was the reverse. The resulted in construct pIMBB864.

Construction of M23 (SecY³⁵⁸PG³⁵⁹/AA) was constructed using Quick-Change PCR site-directed Mutagenesis (Stratagene) using *PfuUltraTM* High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X468(5- TTT GTA CCA GGA ATT CGT GCG GCA GAG CAA ACG GCG AAG TAT-3)was the forward mutagenic primer and X469 (5- ATA CTT CGC CGT TTG CTC TGC CGC ACG AAT TCC TGG TAC AAA-3) was the reverse. This resulted in construct pIMBB865.

Construction of M13 (SecY³⁶⁴KYID³⁶⁷/AAAA): SecY³⁶⁴KYID³⁶⁷/AAAA was constructed by “megaprimer” PCR using pET610 as template and X360 (5' CGTCCGGGAGAGCAAACGGCGG**CGGCGCCGCC**AAAGTAATGACCCGCCTGACCC3') as forward mutagenic primer and X339 (5' GTATTCGCACCCATGGTCTGTTTCC3') as reverse primer. The 0.3kb PCR product was used in a second step PCR with X331 (5' CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB593.

Construction o fM24 (SecY³⁷¹RL³⁷²/AA): SecY³⁷¹RL³⁷²/AA was constructed using Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltraTM* High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X470 (5'- GTC GAC AAA GTA ATG ACC GCC GCG ACC CTG GTT GGT GCG CTG-3') was the forward mutagenic primer and X471 (5'- CAG CGC ACC AAC CAG GGT CGC GGC GGT CAT TAC TTT GTC GAC-3') was the reverse. This resulted in construct pIMBB866.

Construction of M25 (SecY^{T³⁷³}/A): SecY^{T³⁷³}/A was constructed USING Quick-Change PCR site-directed mutagenesis (Stratagene) using *PfuUltraTM* High Fidelity DNA polymerase. In this mutagenesis scheme, plasmid pET610 was used as template, X472 (5'- GTA ATG ACC CGC CTG GCC CTG GTT GGT GCG CTG-3') was the forward

mutagenic primer and X473 (5'-CAG CGC ACC AAC CAG GGC CAG GCG GGT CAT TAC-3') was the reverse. This resulted in construct pIMBB867.

Construction of M15 (SecY⁴²⁸QYE⁴³⁰/AAA): SecY⁴²⁸QYE⁴³⁰/AAA was constructed by “megaprimer” PCR using pET610 as template and X356 (5' GCAAACCTCTGATGATGTCCAGT**GCGGCCGCGT**TCTGCATTGAAGAAGGCGAACC 3') as forward mutagenic primer and X355 (5' GTTAAGAGCGCGACACCACCCGCTGC 3') as reverse primer. The 0.27kb PCR product was used in a second step PCR with X331 (5' CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB602.

Construction of M16 (SecY⁴³⁷NLK⁴³⁹/AAA): SecY⁴³⁷NLK⁴³⁹/AAA was constructed by “megaprimer” PCR using pET610 as template and X354 (5' GAGTCTGCATTGAAGAAGGCG**GCGGCCGCA**GGCTACGGCCGATAAATCGATAG3') as forward mutagenic primer and X355 (5' GTTAAGAGCGCGACACCACCCGCTGC 3') as reverse primer. The 0.25kb PCR product was used in a second step PCR with X331 (5' CACACAGGAAACAGACCATGCATCACCATCAC3') as forward primer. The final 1.4kb PCR product was digested with NcoI and inserted in the corresponding sites of pET610 resulting in pIMBB595.

References

- Beck, K., Wu, L. F., Brunner, J., and Muller, M. (2000). Discrimination between SRP- and SecA/SecB-dependent substrates involves selective recognition of nascent chains by SRP and trigger factor. *Embo J* 19, 134-143.
- Karamanou, S., Sianidis, G., Gouridis, G., Pozidis, C., Papanikolau, Y., Papanikou, E., and Economou, A. (2005). Escherichia coli SecA truncated at its termini is functional and dimeric. *FEBS Lett* 579, 1267-1271.
- Papanikou, E., Karamanou, S., Baud, C., Frank, M., Sianidis, G., Keramisanou, D., Kalodimos, C. G., Kuhn, A., and Economou, A. (2005). Identification of the preprotein binding domain of SecA. *J Biol Chem* 280, 43209-43217.
- van der Does, C., Manting, E. H., Kaufmann, A., Lutz, M., and Driessen, A. J. (1998). Interaction between SecA and SecYEG in micellar solution and formation of the membrane-inserted state. *Biochemistry* 37, 201-210.