Chatzi et al., https://doi.org/10.1083/jcb.201609022

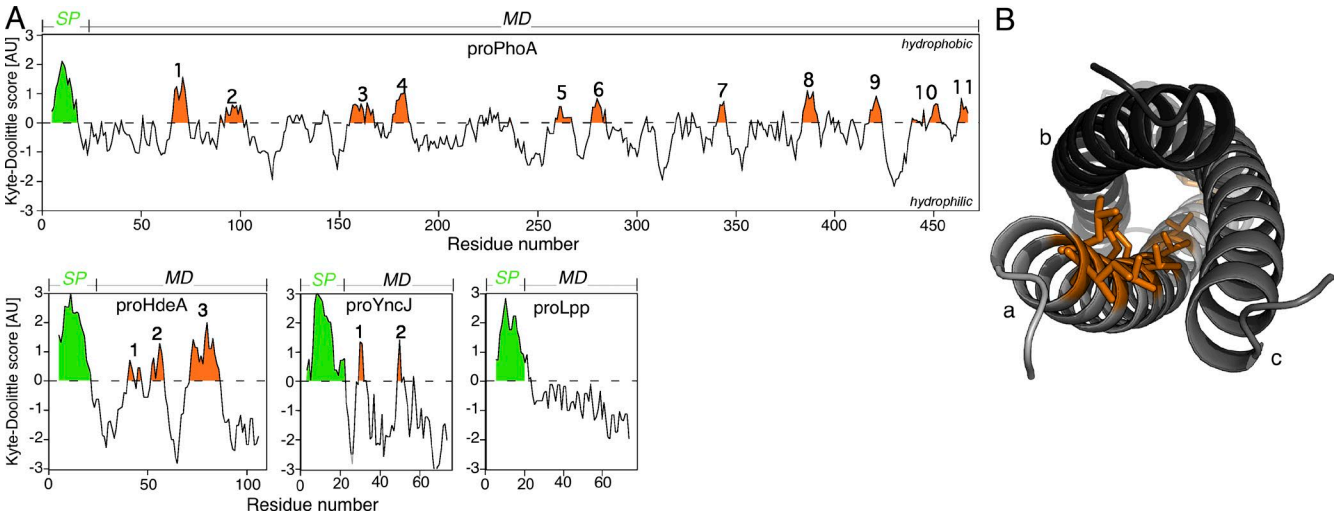

Figure S1. **HPs in preprotein mature domains can be linear or 3D (related to Fig. 1).** (A) Hydrophobicity plots for proPhoA, proHdeA, and proLpp by using Protscale (http://web.expasy.org/protscale/) and a 9-aa window. For visualization purposes, proYncJ is shown using a 5-aa window. Residues corresponding to the signal peptide (SP) or mature domain (MD) of each protein are indicated. The HPs in the mature domain region of each protein are indicated (orange) and numbered (similarly to Fig. 1, B and D). The first HP in YncJ has the lowest hydrophobicity value within our experimental set, yet it is important for targeting (Fig. 1 D). This value was used to set the hydrophobicity threshold of what was defined as a "hydrophobic patch used for targeting" (see Materials and methods section Bioinformatics approach to define hydrophobic patches on proteins) and therefore determines a functional MTS. proLpp mature domain has no detectable linear HPs. The determined HPs were experimentally verified as functional MTSs by hydrophobicity-reducing mutations; PhoAM1, I67A/I68T/L69A/L70T/I71A; PhoAM2, F93A/F94A/I97A/L100A/L102A; PhoAM8-11, L385A/V386T/I387A/V388T/V419T/M420T/V421T/M422A/Y424T/L439A/I441T/Y444T/V451A/V452T/L454T/F461A/Y462T/A466T/A467T/L4668T/L470T; HdeA(noMTS), F42A/L43T/V45A/F49T/V54T/F56T/L60A/V70T/LV1T/V73A/I76T/V79A/I83T/V84A; YncJ(noMTS), F29T/V30A/W31T/V32T/V35A/L48A/V51T). (B) View of the native Lpp trimer (PDB: 1EQ7) along its longitudinal axis. The hydrophobic amino acids of each helix (highlighted in orange; as in Fig. 1 E) face inwards in the trimer core and thus are shielded from solvent. The 3D MTS of Lpp was verified as functional by hydrophobicity-reducing mutations (I27A/L30A/V34A/L37A/V41A/L44A/V48A/V55A/L69A, hereafter Lpp(noMTS)).

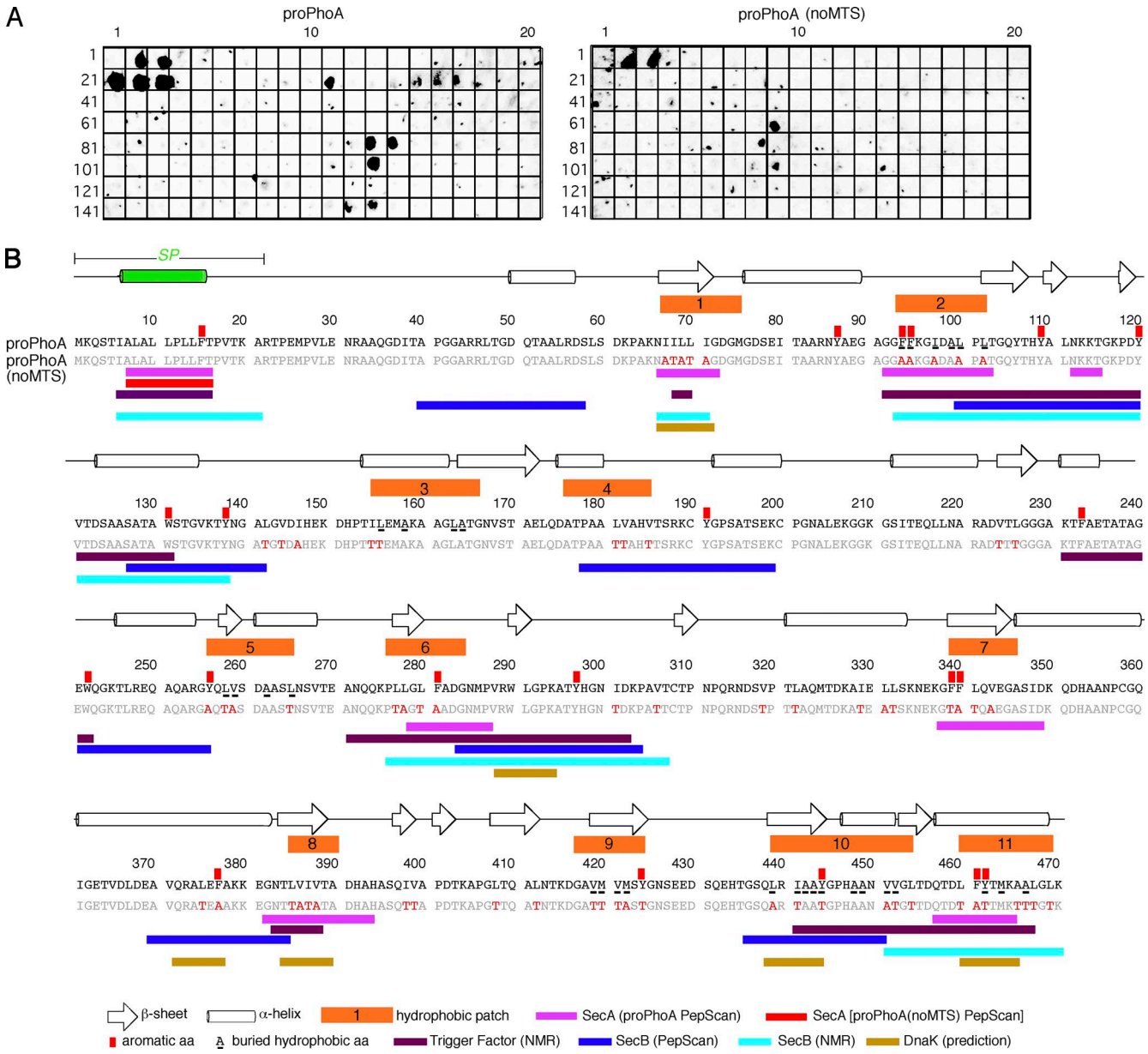

Figure S2. **proPhoA MTSs are required for mature domain targeting (related to Figs. 1 and 3).** (A) SecA binding to proPhoA (left) or proPhoA(noMTS) (right) using 13-residue peptide arrays with 10-residue overlap (for the identity of peptides, see Table S9, in combination with B). proPhoA(noMTS) was designed and used only in PepScans. A representative experiment, after immunostaining with α-SecA antibody (1:50,000 dilution), is shown; $n = 6$. When using the peptide array of proPhoA(noMTS), only binding of SecA on the signal peptide is retained. (B) SecA and chaperone binding sites on proPhoA. The proPhoA primary sequence and secondary structure are shown; residues that were mutated (as indicated) in proPhoA(noMTS) are colored red. Below them, the binding sites for the following chaperones are colored as indicated: trigger factor, as determined by nuclear magnetic resonance (Saio et al., 2014); SecB, as determined by PepScan analysis (Knoblauch et al., 1999) and nuclear magnetic resonance (Huang et al., 2016); DnaK, as predicted by the Limbo server (http://limbo.switchlab.org/); and SecA, as determined by PepScan analysis in the present study (A). Aromatic residues that were proposed to be important for chaperone interactions (Patzelt et al., 2001) are indicated. Hydrophobic amino acids that are buried based on the crystal structure of PhoA (PDB: 3BDG) are underlined. Soluble SecA binds on the signal peptide (SP) and to six more mature domain HPs. Some mature domain HPs might also be recognized by chaperones.

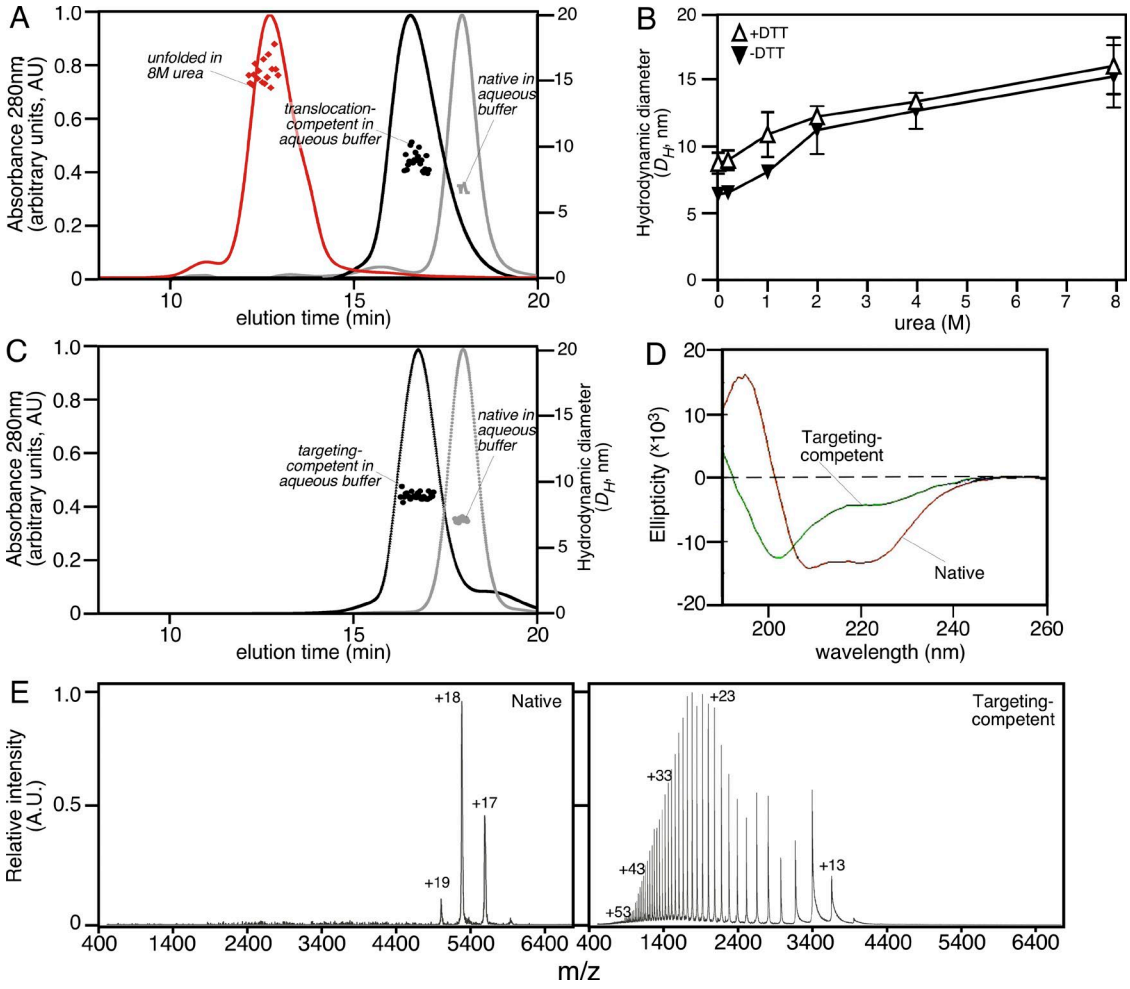

Figure S3. **Biophysical characterization of the proPhoA and PhoA targeting-competent state (related to Figs. 1 and 3).** (A) Representative gel permeation chromatography coupled to multiangle and quasielastic light scattering experiments for proPhoA under native (gray, no urea; no DTT), translocation-competent (black, no urea; 1 mM DTT) and strong denaturing (red, 8 M urea; 1 mM DTT) conditions; UV traces (left y axis, A_{280} arbitrary units) are shown as a function of time (x axis, minutes); $n > 3$. For the native species, natively purified proPhoA was diluted and chromatographed in buffer L. For the translocation-competent and the completely unfolded species, proPhoA purified in 6 M urea was preincubated with 10 mM DTT (30 min; ice), was chromatographed (and buffer exchanged during the chromatography) in buffer L supplemented with the indicated urea and DTT concentration. Protein concentrations after chromatography were in the range of 50–100 µM, anticipated by a 10-fold protein dilution on the column (0.5 mM protein loaded). The hydrodynamic diameters (right y axis, D_H, nanometers) of natively folded monomeric proPhoA (gray squares), translocation-competent proPhoA (black circles), and fully denatured proPhoA (red diamonds) measured online by quasielastic light scattering are shown; mass measurements are not depicted. By default, the translocation-competent proPhoA is also targeting competent. (B) Summary of quasielastic light scattering measurements of the proPhoA hydrodynamic diameter (y axis; D_H, nanometers), derived from experiments similar to those shown in A, under oxidizing (–DTT) or reducing conditions (+DTT) as a function of urea concentration (x axis). For measurements in 0–0.2 M urea, ±DTT, n = 10–15; for all other urea concentration points ±DTT, n = 3–6; SDs are given as error bars. The targeting/translocation-competent proPhoA is the reduced form at 0–0.2 M urea. (C) Representative gel permeation chromatography coupled to multiangle and quasielastic light scattering experiments of PhoA under native (gray, no urea; no DTT) and targeting-competent (black, no urea; 1mM DTT) conditions; n = 3. UV traces (left y axis, A_{280} arbitrary units) are shown as a function of time (x axis, minutes). For the native species, natively purified PhoA was diluted and chromatographed in buffer L. For the targeting-competent species, urea purified PhoA was preincubated with 10 mM DTT (30 min; ice) before being diluted and chromatographed in buffer L supplemented with 1 mM DTT. The hydrodynamic diameters (right y axis, D_H, nanometers) of natively folded dimeric PhoA (gray circles) and targeting-competent PhoA (black circles), measured online by quasielastic light scattering, are shown; mass measurements are not depicted. The targeting-competent PhoA is also translocation-competent on two conditions: (a) by trans addition of its signal peptide or (b) by using a *prl* translocase (Gouridis et al., 2009). (D) Comparison of two representative circular dichroism spectra recorded for natively folded (dark red; no DTT) and targeting-competent (green; 1 mM DTT) PhoA. x axis: wavelength (nanometers); y axis: ellipticity. For the targeting-competent species, urea purified PhoA, preincubated with 10 mM DTT (30 min; ice) was dialyzed (5 liters; 15 h; 4°C) in buffer U supplemented with 8 M urea and 1 mM DTT. For the natively folded species, natively purified PhoA was dialyzed in 5 liters buffer U (15 h; 4°C). Both PhoA species were diluted in buffer U supplemented with 1 mM EDTA; 0.2 M urea; DTT (as indicated) and spectra were recorded. As seen with the corresponding proPhoA species (Fig. 3 B), natively folded PhoA exhibits two minima (208 and 222 nm), typical of folded, predominantly α-helical proteins, whereas the targeting-competent PhoA does not. However, if the urea-purified PhoA is dialyzed (5 liters; 15 h) in buffer U in the absence of DTT, it folds and gives spectra similar to the one shown for the natively purified PhoA; similar behavior was observed for proPhoA under the same conditions (not depicted). (E) Representative native nano–electrospray ionization mass spectrometry spectra of native and targeting-competent PhoA; n = 3. Targeting-competent PhoA acquires many charges with broad distribution, typical of unfolded proteins with increased solvent accessible surface area (Testa et al., 2013) and has a mass of 48.4 kD, consistent with that of a monomer, whereas native PhoA acquires few charges with narrow distribution, typical of well-folded, compact proteins, and has a mass of 96.6 kD, consistent with that of a dimer.

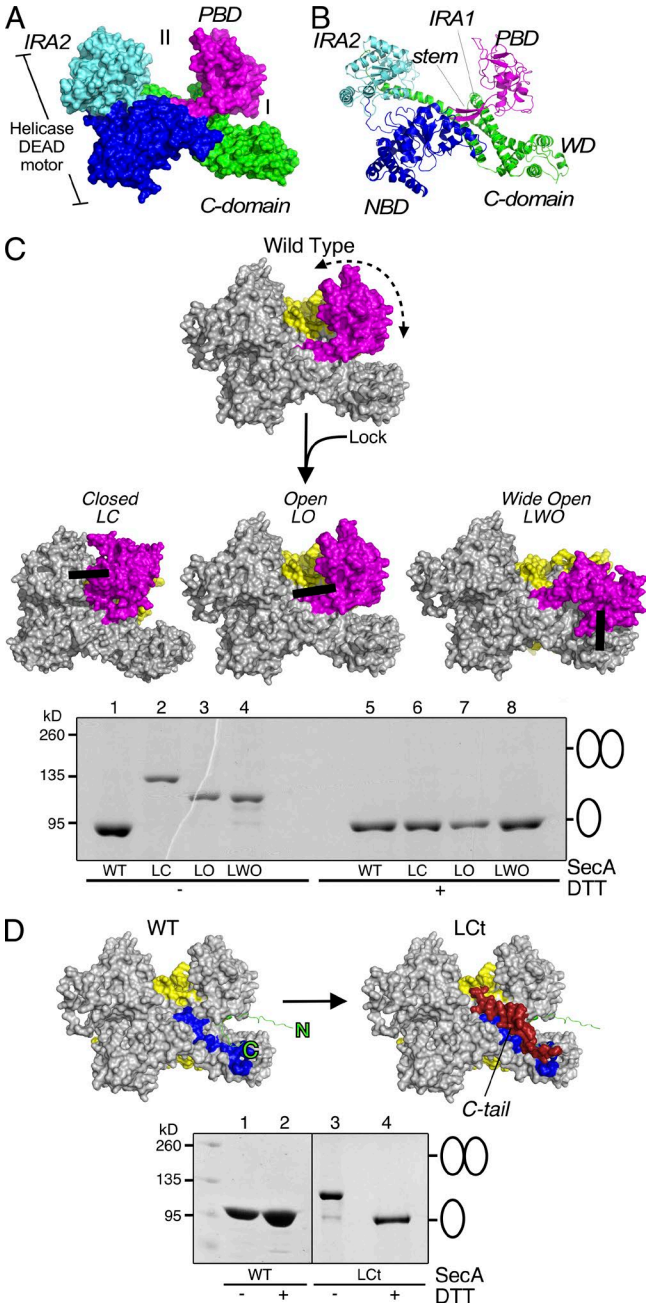

Figure S4. **PBD motions and purification of SecA with immobilized PBD domain or C-tail (related to Fig. 4).** (A and B) Surface (A) and ribbon (B) models of the *E. coli* SecA (PDB: 2FSF) in the open PBD conformation (Papanikolau et al., 2007). The four domains of SecA are NBD (blue) and IRA2 (cyan) that form the helicase DEAD motor, PBD (purple), and the C-domain (green). Stem: the antiparallel β-sheet that connects the PBD to the NBD. Two apparent clamps that form as PBD swivels are indicated (I and II; see also Fig. 4). (C) Schematic presentation of the swiveling flexibility as well as the immobilization of the PBD domain of SecA in three conformational states using engineered disulfide bonds (top). Cysteines at positions K268C/I597C lock SecA in the closed conformation (LC), P301C/S809C cysteines lock SecA in the open conformation (LO), and P301C/Q830C cysteines lock SecA in the wide open conformation (LWO). SecYEG is shown in yellow. Nonreducing SDS-PAGE of the indicated purified Locked SecA derivatives (bottom). Proteins were visualized by Coomassie R-250 staining. Purified His_6SecA(6–834) (K268C/I597C; LC), His_6SecA(6–834) (C98A/P301C/S809C; LO) and His_6SecA(6–834) (C98A/P301C/Q830C; LWO) were analyzed by nonreducing SDS-PAGE on a 7.5% wt/vol acrylamide gel. Under nonreducing conditions (lanes 2–4), all mutants migrate at an apparent molecular mass that is higher than that of the wild type (WT; lane 1). Because none of them have the molecular mass of a dimeric SecA, we concluded that the mutant proteins formed intraprotomeric disulfide bonds and migrated aberrantly during SDS-PAGE as commonly seen before (Mori and Ito, 2006; Karamanou et al., 2007). When a reducing agent is added, aberrant migration is abolished (lane 6–8) and all proteins migrate to the same position as that of the non–cross-linked protein (lane 5). (D) Schematic presentation of C-tail immobilization on SecA (top; blue, PatchA; dark red, SecA C-tail; yellow, SecYEG; green, signal peptide) using engineered disulfide bonds. Intraprotomeric cysteine oxidation of residues M191C/R850C locked the C-tail on SecA (i.e., SecA(LCt)). Purified His_6SecA(6–901) (M191C/R850C; LCt) protein was analyzed on a 7.5% wt/vol acrylamide nonreducing SDS-PAGE and visualized by Coomassie R-250 staining (bottom). Under nonreducing conditions (lane 3), SecA(LCt) migrates at an apparent molecular mass that is higher than the wild type (lane 1). Because it does not have the molecular mass of a dimeric SecA, we concluded that the mutant proteins formed intraprotomeric disulfide bonds. When a reducing agent is added, aberrant migration is abolished (lane 4).

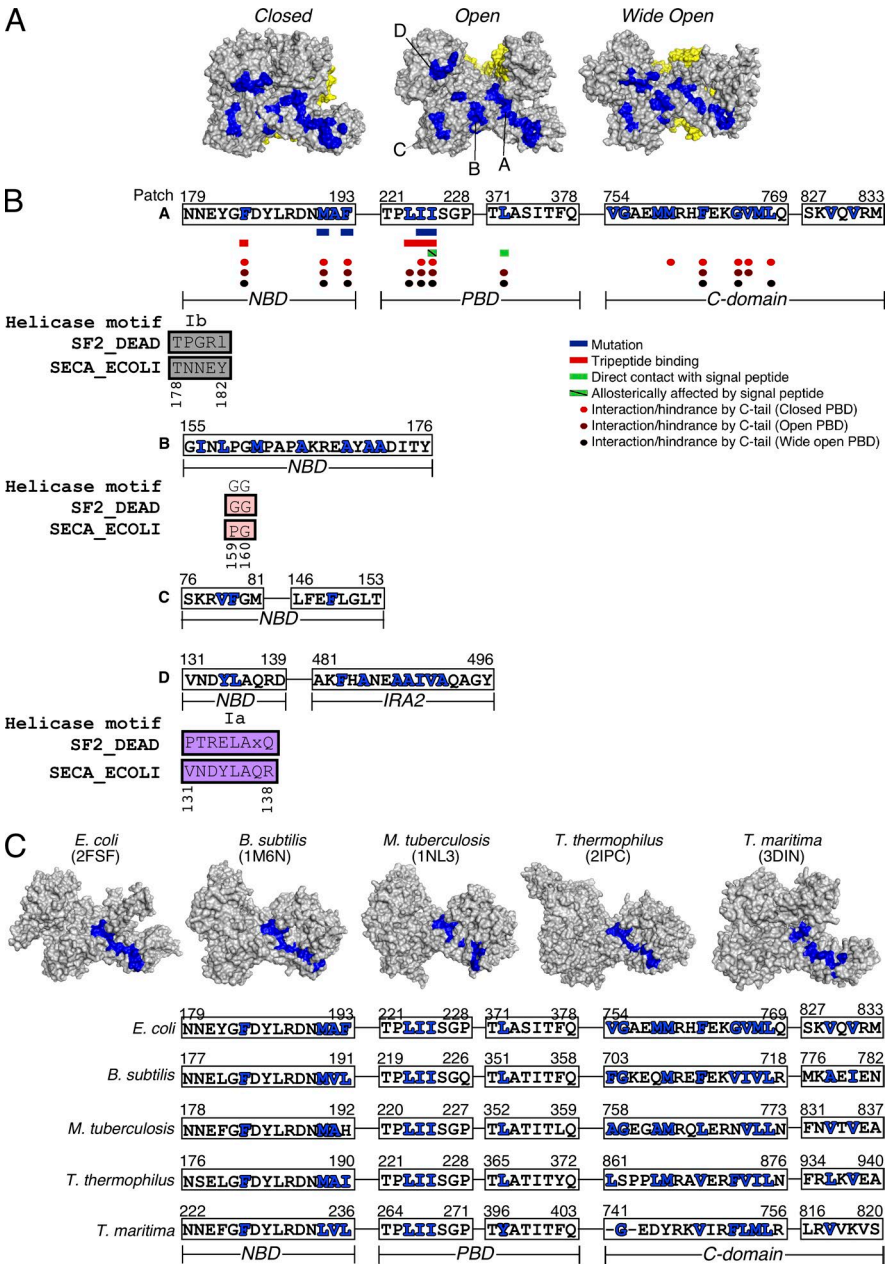

Figure S5. **Hydrophobic patches on SecA (related to Fig. 4).** (A) *E. coli* SecA models with their PBD in three distinct conformational states. The cytoplasmic face of SecA contains four patches of hydrophobic amino acids (blue; indicated as A–D) that are accessible in all PBD positions. Hence, all these potential mature domain–binding sites remain available irrespective of the PBD position. (B) The amino acids of each patch (highlighted in blue) are not next to each other in the linear polypeptide chain but come in close proximity in the 3D space and form continuous hydrophobic patches. Some of the conserved sequences, indicated in color below the Patch sequence, are characteristic DEAD RNA helicase superfamily 2 motifs (Papanikou et al., 2007) known to interact with the oligonucleotide substrate and convey allosteric cross talk to the ATPase machinery. Mutation of four PatchA residues (indicated by a blue bar under the PatchA sequence) to alanyl residues in this study (SecA PatchA) disturbs the hydrophobicity continuity of PatchA (Fig. 4 G) and consequently impacts mature domain binding and preprotein secretion (Fig. 4, H–J). Four PatchA residues (indicated by a red bar) were shown to interact with a co-crystalized tripeptide (Zimmer et al., 2009). Direct and indirect (allosteric) contacts with a signal peptide in solution (Gelis et al., 2007) are indicated (see index; see also Fig. S6 D for structural details). PatchA residues that become completely or partially shielded by the SecA C-tail in the closed, open, and wide open PBD states are indicated by red, dark red, and black circles, respectively (see also Fig. S6 E for structural details). For the wide open state, interactions were identified using the *Bacillus subtilis* SecA wide open state (1M6N) as a template. 1M6N is the only available structure in which the C-tail is resolved. For the closed and open PBD states, *E. coli* models were generated for the localization of the C-tail using the *B. subtilis* SecA structure (1M6N) as a template. (C) Conservation of the hydrophobic PatchA (blue patch) on SecA in various organisms. From left to right: *E. coli* SecA (2FSF; Papanikolau et al., 2007), *B. subtilis* SecA (1M6N; Hunt et al., 2002), *Mycobacterium tuberculosis* SecA (1NL3; Sharma et al., 2003), *Thermus thermophilus* SecA (2IPC; Vassylyev et al., 2006), and *T. maritima* SecA (3DIN; Zimmer et al., 2008). The alignment of the PatchA residues for all SecAs is shown below. Most of these residues are highly conserved or have conserved hydrophobicity.

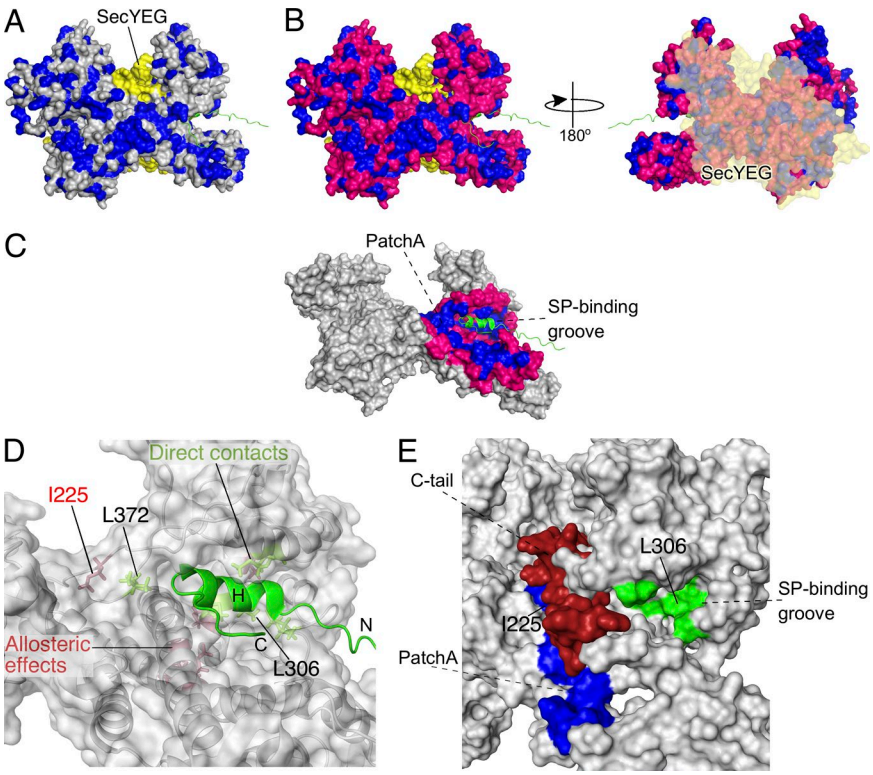

Figure S6. **SecA surface features and detailed interactions with signal peptide and C-tail (related to Fig. 4).** (A) The cytoplasmic platform of SecA (*E. coli* SecA model; as in Fig. S5 A) is enriched in nonpolar amino acids (blue; alanine, glycine, methionine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, and tryptophan). Continuous nonpolar grooves form the SecA hydrophobic patches that are proposed to be involved in binding mature domain signals (Fig. S5, A and B). (B) Polar/charged (pink) and nonpolar (blue) residues are indicated on the cytoplasmic SecA platform (left) and on the SecYEG-interacting surface of SecA (right). Only the cytoplasmic SecA platform is enriched in extensive nonpolar islands, supporting its engagement in interactions with preprotein mature domains. These hydrophobic islands, namely the SecA hydrophobic patches, are outlined by polar and charged residues that may assist MTS binding via electrostatic contacts or hydrogen bonding with polar and charged mature domain residues that surround MTSs. In contrast, polar/charged islands dominate the SecA–SecYEG interface, enabling efficient SecA docking on the mainly charged/polar cytoplasmic protrusions of SecY. (C) Non-polar (blue) and polar/charged (pink) residues are highlighted only at the proximity of the signal peptide-binding groove of SecA (PDB: 2VDA). PatchA of SecA appears as a physical continuation of the signal peptide–binding groove in an orthogonal configuration. The mainly polar C-terminal region of the engaged signal peptide lies on a polar SecA path that connects to PatchA. (D) PatchA and the signal peptide–binding site on SecA are adjacent, but not overlapping. They converge at a 90° angle, forming a characteristic L shape. The signal peptide–binding site of SecA is mainly located in the groove formed between the PBD and IRA1 domains (Gelis et al., 2007). The signal peptide (green) binds with two main components. (a) Its helical hydrophobic region (H) makes hydrophobic contacts (with M235, V239, I291, I292, M305, and L306; lime green; Gelis et al., 2007). These are the major binding contacts, and signal peptide binding is 6- to 15-fold reduced when residues I304 and L306 are mutated (Gelis et al., 2007; Gouridis et al., 2009). (b) Its positively charged N terminus (N) makes electrostatic contacts (with E289, D293, E294, and E708; not depicted; Gelis et al., 2007). The C-terminal extension of the signal peptide is the mature domain. The presence of the signal peptide-induced additional nuclear magnetic resonance –detected chemical shifts of SecA residues that are not involved in direct contacts with the signal peptide (Gelis et al., 2007). These are attributed to allosteric effects (dark red). A representative example with a strong observed nuclear magnetic resonance shift (Gelis et al., 2007) is the I225 residue of PatchA that lies >8 Å away from the closest atom of the signal peptide, and this effect is purely allosteric. Despite the proximity of PatchA and the signal peptide-binding groove, the L372 comprises the only PatchA residue that appears to directly interact with a signal peptide at the C-terminal region of the signal peptide. As the C region of the signal peptide is flexible, this interaction might be transient, and it is unknown if it occurs in the context of the preprotein, that may alter the configuration of the flexible segment. The in-solution nuclear magnetic resonance structure was performed with the signal peptide alone. (E) The C-tail of SecA occupies the PatchA but only partially occludes the signal peptide-binding site of SecA. The C-tail (dark red) makes close docking interactions with residues F184, M191, F193, L223, I224, I225, L372, F762, G765, and V766 in PatchA (blue; I225 is indicated and is buried under the C-tail; interactions were determined in *E. coli* SecA at the open state modeled based on the *B. subtilis* SecA structure (1M6N) for the localization of the C-tail; see also Fig. S5 B). Instead, the bound C-tail essentially hovers over the signal peptide cleft (green). It passes near residues that would be occupied by the C terminus of the signal peptide (see D; e.g., L372). The signal peptide cleft residues (e.g., L306) remain unhindered for interaction with the signal peptide. Other than residues close to L372 of SecA, all other residues that interact with the C-tail mostly surround but are not directly inside the signal peptide–binding groove (Gelis et al., 2007). Deletion of the C-tail can lead to fourfold increased affinity of the PhoA signal peptide for SecA in solution (Gelis et al., 2007). The reduction in signal peptide affinity when the C-tail is bound to soluble SecA is mainly because of the reduction of accessibility to the signal peptide groove rather than direct occlusion of residues.

Table S1. **Secretory preproteins with weak or no apparent extensive hydrophobic patches**

Entry name (UniProt)	Entry accession (UniProt)	Gene name	Signal peptide length (residues)	Maximum K-D hydrophobicity
YBGS_ECOLI	P0AAV6	*ybgS*	24	0.644
YDCA_ECOLI	P0ACW4	*ydcA*	20	0.933
YIFL_ECOLI	P0ADN6	*yifL*	19	0.589
YGIW_ECOLI	P0ADU5	*ygiW*	20	0.744
YHHA_ECOLI	P0ADX7	*yhhA*	17	0.744
HDEB_ECOLI	P0AET2	*hdeB*	29	1.056
PSIF_ECOLI	P0AFM4	*psiF*	21	0.667
MLIC_ECOLI	P28224	*mliC*	17	0.533
ASR_ECOLI	P36560	*asr*	21	–0.044
YQJC_ECOLI	P42616	*yqjC*	20	0.2
YNCJ_ECOLI	P64459	*yncJ*	22	0.122
YHDU_ECOLI	P64619	*yhdU*	30	0.667
YGDI_ECOLI	P65292	*ygdI*	19	–0.111
YGDR_ECOLI	P65294	*ygdR*	19	0.367
PLIG_ECOLI	P76002	*pliG*	22	0.356
YFGI_ECOLI	P76573	*yfgI*	19	0.456
YDDL_ECOLI	P77519	*yddL*	21	0.422
SPY_ECOLI	P77754	*spy*	23	0.389
YICS_ECOLI	Q2M7X4	*yicS*	21	0.833
YJDP_ECOLI	Q6BEX5	*yjdP*	22	0.711

Maximum hydrophobicity values of the Kyte–Doolitle hydrophobic profile (window: 9, linear weight variation model) of *E. coli* secretory preproteins that show weak or no apparent extensive linear hydrophobic patches, following a secretome-wide analysis. Apart from proLpp (Fig. S1, A and E), 19 more preproteins have no apparent prominent linear hydrophobic patches in their primary sequence. Most of their hydrophobicity values are lower than the one that defines a hydrophobic patch capable of acting as an MTS. The weakest such MTS signal was defined experimentally for YncJ (Figs. 1D and S1A and Materials and methods section Bioinformatics approach to define hydrophobic patches on proteins). These proteins are candidates for possessing 3D MTS signals. Three of these proteins, PliG (PDB: 4DY3), YgiW (PDB: 1NNX), and YgdR (PDB: 3FIF), have available crystal structures. We examined whether their mature domains might have 3D, noncontinuous hydrophobic recognition signals like those of Lpp (Fig. 1 E). In YgiW and PliG, there are hydrophobic surfaces created by amino acids on a β-sheet that could potentially also be recognized. YgdR only has very short hydrophobic surfaces of 2 aa. K-D, Kyte–Doolitle.

Table S2. **Buffers used in this study**

Buffer	Composition
Buffer A	50 mM Tris-Cl, pH 8.0, 0.50 M NaCl, 10% glycerol vol/vol, 5 mM imidazole
Buffer B	50 mM Tris-Cl, pH 8.0, 0.50 M NaCl, 10% glycerol vol/vol, 8 M urea, 5 mM imidazole
Buffer C	50 mM Tris-Cl, pH 8.0, 0.50 M NaCl, 10% glycerol vol/vol, 6 M urea, 5 mM imidazole
Buffer D	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 10% glycerol vol/vol, 6 M urea, 5 mM imidazole
Buffer E	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 10% glycerol vol/vol, 6 M urea, 100 mM imidazole
Buffer F	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 6 M urea, 10% glycerol vol/vol
Buffer G	50 mM Tris-Cl, pH 8.0, 1 M NaCl, 10% glycerol vol/vol, 5 mM imidazole
Buffer H	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 10% glycerol vol/vol, 5 mM imidazole
Buffer I	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 10% glycerol vol/vol, 100 mM imidazole
Buffer J	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 10% glycerol vol/vol
Buffer K	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 50% glycerol vol/vol
Buffer L	50 mM Tris-Cl, pH 8.0, 50 mM NaCl
Buffer M	50 mM Tris-Cl, pH 8.0, 1 M NaCl
Buffer N	50 mM Tris-Cl, pH 8.0, 20% glycerol vol/vol, 10 mg/ml DNaseI, 50 mg/ml RNase, 1 mM PMSF
Buffer O	50 mM Tris-Cl pH 8.0
Buffer P	50 mM Tris-Cl, pH 8.0, 20% glycerol vol/vol
Buffer Q	50 mM Tris-Cl, pH 8.0, 0.2 M sucrose
Buffer R	50 mM Tris-Cl, pH 8.0, 50 mM KCl, 5 mM $MgCl_2$
Buffer S	50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 6 M Urea, 1 mM DTT, 1 mM EDTA
Buffer T	50 mM Tris-Cl, pH 8.0, 50 mM KCl, 5 mM $MgCl_2$, 1 mg/ml BSA, 1 mM DTT
Buffer U	5 mM MOPS, pH 7.5, 5 mM NaCl

Table S3. ***E. coli* host strains used in this study**

Strain	Description	Reference or source
DH5α	*fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Invitrogen
JM109	*endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F' traD36 proAB+ lacIq lacZΔM15] hsdR17($r_K^-m_K^+$)*	Promega
BL21(DE3)	*(F- ompT gal dcm lon hsdS_B(r_B^- m_B^-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*	Studier et al., 1990
BL21.19(DE3)	*secA13 (Am) supF (Ts) trp (Am) zch::Tn10 recA::cat clpA::kan)*	Mitchell and Oliver, 1993
BL31(DE3)	A BL21.19 spontaneous mutant derivative that can grow at high temperatures	This study
MC4100	F- araD139 ¢(argF-lac)U169 rpsL150 (Str^R) relA1 flbB5301 deoC1 pstF25 rbsR	Casadaban, 1976

Table S4. **Cloning vectors used in this study**

Vector	Antibiotic resistance	Reference or source
pET5	Ampicillin	Studier and Moffatt, 1986
pET22b+	Ampicillin	EMD Millipore
pET16b	Ampicillin	EMD Millipore
pBAD33	Chloramphenicol	Guzman et al., 1995
pBAD501	Gentamycin[a]	This study

[a]The gentamycin resistance gene was amplified by PCR using the Gem^R plasmid pFASTBAC (Takara Bio, Inc.; a gift from T. Pugsley, Pasteur Institute, Paris, France) as a template and primers X1926 and X1927 and, following MscI–ScaI digestion, replaced the chloramphenicol resistance gene on a pBAD33 vector.

Table S5. **Synthetic genes or gene fragments used in this study**

Identity	Gene	Sequence before mutagenesis (5′–3′)	Sequence after mutagenesis (5′–3′)
SG PhoA (350-471) M8-11	*PhoA*	AAACAGGATCATGCTGCGAATCCTTGTGGGCAAATTGGCGAGACGGTC GATCTCGATGAAGCCGTACAACGGGCGCTGGAATTCGCTAAAAAGGAG GGTAACACGCTGGTCATAGTCACCGCTGATCACGCCCACGCCAGCCAG ATTGTTGCGCCGGATACCAAAGCTCCGGGCCTCACCCAGGCGCTAAAT ACCAAAGATGGCGCAGTGATGGTGATGAGTTACGGGAACTCCGAAGAG GATTCACAAGAACATACCGGCAGTCAGTTGCGTATTGCGGCGTATGGC CCGCATGCCGCCAATGTTGTTGGACTGACCGACCAGACCGATCTCTTC TACACCATGAAAGCCGCTCTGGGGCTGAAATAA	AAACAGGATCATGCTGCGAATCCTTGTGGGCAAATTGGCGAGACG GTCGATCTCGATGAAGCCGTACAACGGGCGACCGAAGCGGCT AAAAAGGAGGGTAACACGGCGACCGCGACCACCGCTGATCAC GCCCACGCCAGCCAGACCACCGCGCCGGATACCAAAGCTCCG GGCACCACCCAGGCGACCAATACCAAAGATGGCGCAACCACCACC GCGAGTACCGGGAACTCCGAAGAGGATTCACAAGAACATACCGGC AGTCAGGCGCGTACCGCGGCGACCGGCCCGCATGCCGCCAATGCG ACCGGAACCACCGACCAGACCGATACCGCGACCACCATGAAAACC ACCACCGGGACCATG
SG HdeA (noMTS)	*HdeA*	AAAAAAGTATTAGGCGTTATTCTTGGTGGTCTGCTTCTTCTGCCAGTT GTGAGCAATGCAGCGGATGCGCAAAAAGCAGCTGATAACAAAAAACCG GTCAACTCCTGGACCTGTGAAGATTTCCTGGCTGTGGACGAATCCTTC CAGCCAACTGCAGTTGGTTTTGCTGAAGCGCTGAACAACAAAGATAAA CCAGAAGATGCGGTTTTAGATGTTCAGGGTATTGCAACCGTAACCCCA GCTATCGTTCAGGCTTGTACTCAGGATAAACAAGCCAACTTTAAAGAT AAAGTTAAAGGCGAATGGGACAAAATTAAGAAAGATATGTAA	AAAAAAGTATTAGGCGTTATTCTTGGTGGTCTGCTTCTTCTGCCA GTTGTGAGCAATGCAGCGGATGCGCAAAAAGCAGCTGATAACAAA AAACCGGTCAACTCCTGGACCTGTGAAGATGCGACCGCTGCGGAC GAATCCACCCAGCCAACTGCAACCGGTACCGCTGAAGCGGCGAAC AACAAAGATAAACCAGAAGATGCGACCACCGATGCGCAG GGTACCGCAACCGCGACCCCAGCTACCGCGCAGGCTTGTACTCAG GATAAACAAGCCAACTTTAAAGATAAAGTTAAAGGCGAATGGGAC AAAATTAAGAAAGATATGATG
SG YncJ (noMTS)	*YncJ*	TTTACGAAGGCGTTATCGGTTGTCTTATTAACGTGTGCTCTGTTTTCA GGACAACTCATGGCAGGGCACAAAGGACATGAATTTGTGTGGGTAAAG AATGTGGATCATCAGCTGCGTCATGAAGCGGACAGCGATGAATTGCGT GCTGTGGCGGAAGAGTCGGCGGAAGGTTTGCGCGAGCATTTTTACTGG CAAAAATCGCGCAAACCAGAAGCGGGACAACGTTGA	TTTACGAAGGCGTTATCGGTTGTCTTATTAACGTGTGCTCTGTTT TCAGGACAACTCATGGCAGGGCACAAAGGACATGAAACCGCG ACCACCAAGAATGCGGATCATCAGCTGCGTCATGAAGCGGACAGC GATGAAGCGCGTGCTACCGCGGAAGAGTCGGCGGAAGGTTTGCGC GAGCATTTTTACTGGCAAAAATCGCGCAAACCAGAAGCGGGACAA CGTATGATG

The following genes or gene fragments (as indicated) carrying multiple mutations (shown in bold) were produced by Integrated DNA Technologies (IDN) and delivered as pUCIDT(Amp) clones. The NdeI–XhoI restriction sites (underlined) were subcloned in pET22b.

Table S6. **Genetic constructs used in this study**

Gene	UniProt KB accession number	Plasmid name	Vector	Cloning/PCR strategy or source
Preproteins and their derivatives				
proBglX	P33363 (proBglX)	pIMBB1036	pET22b	Gouridis et al., 2009
BglX	P33363 (proBglX)	pIMBB1037	pET22b	Gouridis et al., 2009
proBglX(1-132)	P33363 (proBglX)	pIMBB1229	pET22b	The fragment amplified from pIMBB1036 using X732 and X994 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
BglX(21-132)	P33363 (proBglX)	pIMBB1230	pET22b	The fragment amplified from pIMBB1036 using X734 and X994 primers, was NdeI–XhoI digested and cloned to the corresponding vector sites
proAmy1	P25718 (proAmy1)	pIMBB1044	pET22b	Gouridis et al., 2009
Amy1	P25718 (proAmy1)	pIMBB1045	pET22b	Gouridis et al., 2009
proAmy1(1-131)	P25718 (proAmy1)	pIMBB1227	pET22b	The fragment amplified from pIMBB1044 using X744 and X995 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
Amy1(18-131)	P25718 (proAmy1)	pIMBB1228	pET22b	The fragment amplified from pIMBB1044 using X746 and X995 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA	P00634 (proPhoA)	pIMBB882	pET22b	Gouridis et al., 2009
proPhoA	P00634 (proPhoA)	pIMBB1081	pET22b	The fragment amplified from pIMBB882 using X560 and X807 primers was NdeI–HindIII digested and replaced the corresponding fragment in pIMBB1082
proPhoA(cys-)	P00634 (proPhoA)	pIMBB977	pET22b	Cysteins were mutated to alanines using the Quick-Change Mutagenesis protocol (Agilent Technologies), pIMBB882 template, and the primer pairs X678/X679, X680/X681, X682/X683, and X684/X685
proPhoA	P00634 (proPhoA)	pIMBB932	pBAD33	Gouridis et al., 2013
proPhoA	P00634 (proPhoA)	pIMBB1570	pBAD501	The KpnI–HindIII proPhoA fragment from pIMBB932 was subcloned into the corresponding sites of pBAD501, the HindIII site was destroyed by PCR mutagenesis using the primer pair X1915/X1916I, and the NdeI–XhoI fragment of the resulting plasmid was replaced by the corresponding fragment from pIMBB1081
PhoA	P00634 (proPhoA)	pIMBB1080	pET22b	The fragment amplified from pIMBB882 using X806 and X561 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
PhoA(cys-)	P00634 (proPhoA)	pIMBB1052	pET22b	The 1,347-bp fragment (mature domain without Arg22) was isolated by PCR using template pIMBB977 (proPhoAHis Δcys pET22b) and primers X646 (Forw NdeI) and X561 (Rev XhoI), and the PCR product was digested by NdeI–XhoI and cloned to the same sites of pET22b
proPhoA(1-122)	P00634 (proPhoA)	pIMBB1203	pET22b	The fragment amplified from pIMBB1081 using X560 and X728 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
PhoA(23-122)	P00634 (proPhoA)	pIMBB1183	pET22b	The fragment amplified from pIMBB882 using X806 and X936 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(1-82)	P00634 (proPhoA)	pIMBB1002	pET22b	The fragment amplified from pIMBB882 using X560 and X729 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(1-78)	P00634 (proPhoA)	pIMBB1152	pET22b	The fragment amplified from pIMBB977 using X560 and Ming Tao's (Rev XhoI) primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(1-62)	P00634 (proPhoA)	pIMBB1001	pET22b	Gouridis et al., 2009
proPhoA(1-50)	P00634 (proPhoA)	pIMBB1151	pET22b	The fragment amplified by pIMBB977 using X560 and X928 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(1-40)	P00634 (proPhoA)	pIMBB1150	pET22b	The fragment amplified from pIMBB977 using X560 and X927 primers was NdeI–XhoI digested and cloned to the corresponding vector sites

Table S6. **Genetic constructs used in this study** *(Continued)*

Gene	UniProt KB accession number	Plasmid name	Vector	Cloning/PCR strategy or source
proPhoA(1-30)	P00634 (proPhoA)	pIMBB1149	pET22b	The fragment amplified from pIMBB977 using X560 and X926 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA M1	P00634 (proPhoA)	pIMBB1355	pET22b	Quick Change Mutagenesis PCR System (Agilent Technologies) using pIMBB882 template and the mutagenic primer pairs X1058/X1059 and X1060/X1061
proPhoA(1-122)M1	P00634 (proPhoA)	pIMBB1358	pET22b	The fragment amplified by pIMBB1355 using X560 and X1146 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
PhoA(23-122)M1	P00634 (proPhoA)	pIMBB1364	pET22b	The fragment amplified from pIMBB1358 using X646 and X1146 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA M2	P00634 (proPhoA)	pIMBB1356	pET22b	Quick Change Mutagenesis PCR System (Agilent Technologies) using pIMBB882 template and the mutagenic primer pairs X1062/X1063, X1064/X1065, and X1066/X1067
proPhoA(1-122)M2	P00634 (proPhoA)	pIMBB1359	pET22b	The fragment amplified from pIMBB1356 using X560 and X1146 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
PhoA(23-122)M2	P00634 (proPhoA)	pIMBB1365	pET22b	The fragment amplified from pIMBB1359 using X646 and X1146 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA M1, M2	P00634 (proPhoA)	pIMBB1357	pET22b	Quick Change Mutagenesis PCR System (Agilent Technologies) using pIMBB882 template and the mutagenic primer pairs: X1058/X1059, X1060/X1061, X1062/X1063, X1064/X1065, and X1066/X1067
proPhoA(1-122)M1, M2	P00634 (proPhoA)	pIMBB1360	pET22b	The fragment amplified from pIMBB1357 using X560 and X1146 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
PhoA(23-122) M1,M2	P00634 (proPhoA)	pIMBB1366	pET22b	The fragment amplified from pIMBB1360 using X646 and X1146 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(123-471)	P00634 (proPhoA)	pIMBB1234	pET22b	pIMBB1081 was HindIII–XhoI digested; the vector was isolated and ligated to the HindIII–XhoI fragment that was amplified from pIMBB1081 using X998 and X561 primers
PhoA(123-471	P00634 (proPhoA)	pIMBB1235	pET22b	The fragment amplified from pIMBB882 using X999 and X561 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(250-471)	P00634 (proPhoA)	pIMBB1361	pET22b	pIMBB1081 was HindIII–XhoI digested; the vector was isolated and ligated to the HindIII–XhoI fragment that was amplified from pIMBB1081 using X1068 and X561primers
PhoA(250-471)	P00634 (proPhoA)	pIMBB1434	pET22b	The fragment amplified by colony PCR from BL21.19 strain using X1184 and X561 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(350-471)	P00634 (proPhoA)	pIMBB1362	pET22b	pIMBB1081 was HindIII–XhoI digested; the vector was isolated and ligated to the HindIII–XhoI fragment amplified from pIMBB1081 using X1069 and X561 primers
PhoA(350-471)	P00634 (proPhoA)	pIMBB1435	pET22b	The fragment amplified by colony PCR from BL21.19 strain using X1185 and X561 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proPhoA(350-471)M8-11	P00634 (proPhoA)	pIMBB1532	pET22b	The fragment amplified from pIMBB1531 using X1069 and X1572 primers was HindIII–XhoI digested and replaced the corresponding fragment on pIMBB1081
PhoA(350-471)M8-11	P00634 (proPhoA)	pIMBB1531	pET22b	The NdeI/XhoI digested fragment from IDT vector SG PhoAM8-11 was subcloned to the corresponding vector sites
XXXX-PhoA	P00634	pIMBB1082	pET22b	This construct was created for cloning convenience. A 1.7-kb NdeI–HindIII fragment was cloned to the corresponding sites of pET22b, resulting in pET22b/XXXX-His. To the HindIII–XhoI sites of this construct the MD of PhoA was cloned following digestion by HindIII–XhoI of the PCR fragment amplified from pIMBB882 using primers X781 and X561.
proPpiA	POAFL3 (proPpiA)	pIMBB1042	pET22b	Gouridis et al., 2009
PpiA	POAFL3 (proPpiA)	pIMBB1043	pET22b	Gouridis et al., 2009
proPpiA(1-125)	POAFL3 (proPpiA)	pIMBB1225	pET22b	The fragment amplified from pIMBB1042 using X741 and X993 primers was NdeI–XhoI digested and cloned to the corresponding vector sites

Table S6. **Genetic constructs used in this study** *(Continued)*

Gene	UniProt KB accession number	Plasmid name	Vector	Cloning/PCR strategy or source
PpiA(25-125)	POAFL3 (proPpiA)	pIMBB1226	pET22b	The fragment amplified from pIMBB1042 using X743 and X993 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proSpy	P77754 (proSpy)	pIMBB1331	pET22b	The fragment amplified by colony PCR from BL21.19 *E. coli* strain using X1128 and X1129 primes was NdeI–XhoI digested and cloned to the corresponding vector sites
Spy	P77754 (proSpy)	pIMBB1332	pET22b	The fragment amplified by colony PCR from BL21.19 *E. coli* strain using X1130 and X1129 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proYehR	P33354 (proYehR)	pIMBB1034	pET22b	The fragment amplified by colony PCR from JM109 *E. coli* strain using X771 and X772 primers was NdeI–XhoI digesetd and cloned to the corresponding vector sites
YehR	P33354 (proYehR)	pIMBB1035	pET22b	The fragment amplified by colony PCR from JM109 *E. coli* strain using X773 and X772 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proHdeA	POAES9 (proHdeA)	pIMBB1483	pET22b	The fragment amplified by colony PCR from DH5a *E. coli* strain using X1393 and X1394 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
HdeA	POAES9 (proHdeA)	pIMBB1489	pET22b	The fragment amplified by colony PCR from DH5a *E. coli* strain using X1395 and X1394 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proHdeA(noMTS)	POAES9 (proHdeA)	pIMBB1527	pET22b	The NdeI/XhoI digested fragment from IDT vector SG HdeA(noMTS) was cloned to the corresponding vector sites
HdeA(noMTS)	POAES9 (proHdeA)	pIMBB1528	pET22b	The fragment amplified from the IDT vector SG HdeA(noMTS) using X1395 and X1571 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proLpp	P69776 (proLpp)	pIMBB1321	pET22b	The fragment amplified by colony PCR from BL21.19 *E. coli* strain using X1081 and X1082 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proLpp(C21A)	P69776 (proLpp)	pIMBB1322	pET22b	The mutation C21A was introduced using Quick Change PCR Mutagenesis protocol, pIMBB1321 template, and mutagenic primers X1075 and X1076
proLpp(C21A)(noMTS)	P69776 (proLpp)	pIMBB1425	pET22b	The mutations were introduced using the Quick-Change Mutagenesis protocol, pIMBB1322 template and mutagenic primer pairs X1188/X1189, X1190/X1191, X1192/X1193, X1194/X1195, X1196/X1197, X1198/X1199, X1200/X1201, X1202/1203, and X1204/1205
Lpp(C21A)(noMTS)	P69776 (proLpp)	pIMBB1426	pET22b	The fragment amplified from pIMBB1425 using X1171 and X1082 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proYncJ	P64459 (proYncJ)	pIMBB1485	pET22b	The fragment amplified by colony PCR from DH5a *E. coli* strain using X1422 and X1423 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
YncJ	P64459 (proYncJ)	pIMBB1491	pET22b	The fragment amplified by colony PCR from DH5a *E. coli* strain using X1424 and X1423 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proYncJ(noMTS)	P64459 (proYncJ)	pIMBB1524	pET22b	The NdeI–XhoI digested fragment from IDT vector SG YncJ(noMTS) was cloned to the corresponding vector sites
YncJ(noMTS)	P64459 (proYncJ)	pIMBB1525	pET22b	The fragment amplified from the IDT vector SG YncJ(noMTS) using X1424 and X1570 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proYncJ-PhoA	P64459 (proYncJ)	pIMBB1618	pBAD501	The fragment amplified by colony PCR from *E. coli* strain DH5a using primers X1422 and X1973 was NdeI-HindIII digested and cloned to the corresponding sites of pIMBB1570
proYncJ(noMTS)-PhoA	P64459 (proYncJ)	pIMBB1616	pBAD501	The proYncJ(noMTS) fragment amplified by PCR from pIMBB1524 using primers X1422 and X1973, was NdeI–HindIII digested and cloned to the corresponding sites of pIMBB1570
proOsmB	POADA7 (proOsmB)	pIMBB1024	pET22b	The fragment amplified by colony PCR from JM109 *E. coli* strain using X756 and X757 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
proOsmB(C24A)	POADA7 (proOsmB)	pIMBB1319	pET22b	C24A was introduced by Quick Change PCR Mutagenesis System (Agilent Technologies) using pIMBB1024 template and the mutagenic primers X1048 and X1049
OsmB	POADA7 (proOsmB)	pIMBB1025	pET22b	The fragment amplified by colony PCR from JM109 *E. coli* strain using X758 and X757 primers was NdeI–XhoI digested and cloned to the corresponding vector sites

Table S6. **Genetic constructs used in this study** *(Continued)*

Gene	UniProt KB accession number	Plasmid name	Vector	Cloning/PCR strategy or source
OsmB(C24A)	POADA7 (proOsmB)	pIMBB1320	pET22b	The fragment amplified by colony PCR from BL21.19 *E. coli* strain using X1077 and X757 primers was NdeI–XhoI digested and cloned to the corresponding vector sites
***secA* and derivatives**				
secA(1-901)	P10408 (SecA)	pIMBB10	pET5	Karamanou et al., 1999
secA(6-901)	P10408 (SecA)	pIMBB7	pET5	Karamanou et al., 1999
secA (3cys-) (6-901)	P10408 (SecA)	pIMBB258	pET5	The 2.5-kB NcoI fragment from pT7-7 (a gift from D. Oliver, Wesleyan University, Middletown, CT) replaced the corresponding fragment in pIMBB7
secA(6-901)(M191C/R850C) ***or secA LCt***	P10408 (SecA)	pIMBB987	pET5	The M191C and R850C mutations were introduced using the Quick Change Mutagenesis protocol, pIMBB258 template, and mutagenic primers X706-X707 and X722-X723
secA(9-901)	P10408 (SecA)	pIMBB261	pET16b	The fragment amplified from pIMBB10 using X178 and X131 primers was NdeI–BamHI digested and cloned to the corresponding vector sites
secA(9-834)	P10408 (SecA)	pIMBB552	pET16b	The fragment amplified from pIMBB10 using X272 and X107 primers was KpnI–BamHI digested and cloned to the corresponding sites of pIMBB261
secA(9-834) (Q830C)	P10408 (SecA)	pIMBB796	pET16b	The Q830C mutation was introduced using the Quick Change Mutagenesis Protocol, pIMBB552 template, and mutagenic primers X649-X650
secA(6-834)	P10408 (SecA)	pIMBB798	pET5	The 2019bp AsuI–SspI fragment of pIMBB7 was replaced by the corresponding fragment from pIMBB552
secA(6-834) (C98A)	P10408 (SecA)	pIMBB834	pET5	The C98A mutation was introduced using the Quick Change Mutagenesis protocol, pIMBB798 template, and the mutagenic primers X534 and X535
secA(6-834) (S809C)	P10408 (SecA)	pIMBB808	pET5	The S809C mutation was introduced using the Quick Change Mutagenesis protocol, pIMBB798 template, and the mutagenic primers X434 and X435
secA(6-834) (P301C/S809C)	P10408 (SecA)	pIMBB815	pET5	The P301C mutation was introduced using the Quick Change Mutagenesis protocol, pIMBB808 template, and the mutagenic primers X442 and X443
secA(6-834) (C98A/P301C/S809C) or ***secA LO***	P10408 (SecA)	pIMBB941	pET5	The C98A mutation was introduced using the Quick Change Mutagenesis protocol, pIMBB815 template, and the mutagenic primers X534 and X535
secA(6-834) (Q830C)	P10408 (SecA)	pIMBB799	pET5	The 2019bp AsuI-SspI fragment of pIMBB7 was replaced by the corresponding fragment from pIMBB796
secA(6-834) (P301C/Q830C)	P10408 (SecA)	pIMBB812	pET5	The P301C mutation was introduced using the Quick Change Mutagenesis protocol, pIMBB799 template, and mutagenic primers X442 and X443
secA(6-834) (C98A/P301C/Q830C) or ***secA LWO***	P10408 (SecA)	pIMBB942	pET5	The C98A mutation was introduced using the Quick Change Mutagenesis protocol, pIMBB812 template, and mutagenic primers X534 and X535
secA(6-834) (K268C/I597C) or ***secA LC***	P10408 (SecA)	pIMBB1394	pET5	The K268C and I597C mutations were introduced using the Quick Change Mutagenesis protocol, pIMBB798 template, and mutagenic primers X1032-X1033 and X1040-X1041
secA(6-834) (M191A/F193A)	P10408 (SecA)	pLMB0110	pET16b	The M191A and F193A mutations were introduced using the Quick Change mutagenesis protocol (Agilent Technologies), pIMBB798 template, and the mutagenic primers X1958-X1959
secA(6-834) (M191A/F193A/I224A/I225A) or ***secA PatchA***	P10408 (SecA)	pLMB1666	pET16b	The I224A and I225A mutations were introduced using the Quick Change Mutagenesis PCR System (Agilent Technologies), pLMB0110 template, and the mutagenic primers X1954-X1955
***secYEG* and derivatives**				
secYEG		pET610		A gift from A. Driesssen, University of Groningen, Groningen, Netherlands (van der Does et al., 1996).

Genes were cloned in plasmid vectors using mapped restriction sites (as indicated). Mutations were introduced using protocols, templates, and primers (as indicated). Restriction enzymes, dNTPs, and T4 DNA ligase were either from Minotech (Greece), Promega, or New England Biolabs, Inc. For mutagenesis PCR reactions, PFU Ultra Polymerase (Agilent Technologies) was used; for gene amplification either Expand High fidelity Polymerase (Roche) or DNA *Taq* polymerase (Thermo Fisher Scientific). DpnI was used to cleave the maternal methylated DNA (R0176S; New England Biolabs, Inc.) according to the QuickChange Site-Directed Mutagenesis protocol (http://www.genomics.agilent.com; Agilent Technologies). Plasmids were transformed in DH5α cells. Sequencing was performed by Macrogen.

Provided online are three tables in a PDF. Table S7 shows primers used in this study. Table S8 shows the predicted hydrodynamic radii of secretory proteins that use the Sec secretion system. Table S9 provides the sequences of proPhoA peptides used in the peptide arrays shown in Figs. 1 C and S2 A.

References

Casadaban, M.J. 1976. Transposition and fusion of the lac genes to selected promoters in Escherichia coli using bacteriophage lambda and Mu. *J. Mol. Biol.* 104:541–555. http://dx.doi.org/10.1016/0022-2836(76)90119-4

Gelis, I., A.M. Bonvin, D. Keramisanou, M. Koukaki, G. Gouridis, S. Karamanou, A. Economou, and C.G. Kalodimos. 2007. Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR. *Cell.* 131:756–769. http://dx.doi.org/10.1016/j.cell.2007.09.039

Gouridis, G., S. Karamanou, I. Gelis, C.G. Kalodimos, and A. Economou. 2009. Signal peptides are allosteric activators of the protein translocase. *Nature.* 462:363–367. http://dx.doi.org/10.1038/nature08559

Gouridis, G., S. Karamanou, M.F. Sardis, M.A. Schärer, G. Capitani, and A. Economou. 2013. Quaternary dynamics of the SecA motor drive translocase catalysis. *Mol. Cell.* 52:655–666. http://dx.doi.org/10.1016/j.molcel.2013.10.036

Guzman, L.M., D. Belin, M.J. Carson, and J. Beckwith. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J. Bacteriol.* 177:4121–4130. http://dx.doi.org/10.1128/jb.177.14.4121-4130.1995

Huang, C., P. Rossi, T. Saio, and C.G. Kalodimos. 2016. Structural basis for the antifolding activity of a molecular chaperone. *Nature.* 537:202–206. http://dx.doi.org/10.1038/nature18965

Hunt, J.F., S. Weinkauf, L. Henry, J.J. Fak, P. McNicholas, D.B. Oliver, and J. Deisenhofer. 2002. Nucleotide control of interdomain interactions in the conformational reaction cycle of SecA. *Science.* 297:2018–2026. http://dx.doi.org/10.1126/science.1074424

Karamanou, S., E. Vrontou, G. Sianidis, C. Baud, T. Roos, A. Kuhn, A.S. Politou, and A. Economou. 1999. A molecular switch in SecA protein couples ATP hydrolysis to protein translocation. *Mol. Microbiol.* 34:1133–1145. http://dx.doi.org/10.1046/j.1365-2958.1999.01686.x

Karamanou, S., G. Gouridis, E. Papanikou, G. Sianidis, I. Gelis, D. Keramisanou, E. Vrontou, C.G. Kalodimos, and A. Economou. 2007. Preprotein-controlled catalysis in the helicase motor of SecA. *EMBO J.* 26:2904–2914. http://dx.doi.org/10.1038/sj.emboj.7601721

Knoblauch, N.T., S. Rüdiger, H.J. Schönfeld, A.J. Driessen, J. Schneider-Mergener, and B. Bukau. 1999. Substrate specificity of the SecB chaperone. *J. Biol. Chem.* 274:34219–34225. http://dx.doi.org/10.1074/jbc.274.48.34219

Mitchell, C., and D. Oliver. 1993. Two distinct ATP-binding domains are needed to promote protein export by *Escherichia coli* SecA ATPase. *Mol. Microbiol.* 10:483–497. http://dx.doi.org/10.1111/j.1365-2958.1993.tb00921.x

Mori, H., and K. Ito. 2006. The long alpha-helix of SecA is important for the ATPase coupling of translocation. *J. Biol. Chem.* 281:36249–36256. http://dx.doi.org/10.1074/jbc.M606906200

Papanikolau, Y., M. Papadovasilaki, R.B. Ravelli, A.A. McCarthy, S. Cusack, A. Economou, and K. Petratos. 2007. Structure of dimeric SecA, the *Escherichia coli* preprotein translocase motor. *J. Mol. Biol.* 366:1545–1557. http://dx.doi.org/10.1016/j.jmb.2006.12.049

Papanikou, E., S. Karamanou, and A. Economou. 2007. Bacterial protein secretion through the translocase nanomachine. *Nat. Rev. Microbiol.* 5:839–851. http://dx.doi.org/10.1038/nrmicro1771

Patzelt, H., S. Rüdiger, D. Brehmer, G. Kramer, S. Vorderwülbecke, E. Schaffitzel, A. Waitz, T. Hesterkamp, L. Dong, J. Schneider-Mergener, et al. 2001. Binding specificity of *Escherichia coli* trigger factor. *Proc. Natl. Acad. Sci. USA.* 98:14244–14249. http://dx.doi.org/10.1073/pnas.261432298

Saio, T., X. Guan, P. Rossi, A. Economou, and C.G. Kalodimos. 2014. Structural basis for protein antiaggregation activity of the trigger factor chaperone. *Science.* 344:1250494. http://dx.doi.org/10.1126/science.1250494

Sharma, V., A. Arockiasamy, D.R. Ronning, C.G. Savva, A. Holzenburg, M. Braunstein, W.R. Jacobs Jr., and J.C. Sacchettini. 2003. Crystal structure of Mycobacterium tuberculosis SecA, a preprotein translocating ATPase. *Proc. Natl. Acad. Sci. USA.* 100:2243–2248. http://dx.doi.org/10.1073/pnas.0538077100

Studier, F.W., and B.A. Moffatt. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* 189:113–130. http://dx.doi.org/10.1016/0022-2836(86)90385-2

Studier, F.W., A.H. Rosenberg, J.J. Dunn, and J.W. Dubendorff. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* 185:60–89. http://dx.doi.org/10.1016/0076-6879(90)85008-C

Testa, L., S. Brocca, C. Santambrogio, A. D'Urzo, J. Habchi, S. Longhi, V.N. Uversky, and R. Grandori. 2013. Extracting structural information from charge-state distributions of intrinsically disordered proteins by non-denaturing electrospray-ionization mass spectrometry. *Intrinsically Disord. Proteins.* 1:e25068. http://dx.doi.org/10.4161/idp.25068

Ullers, R.S., D. Ang, F. Schwager, C. Georgopoulos, and P. Genevaux. 2007. Trigger Factor can antagonize both SecB and DnaK/DnaJ chaperone functions in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA.* 104:3101–3106. http://dx.doi.org/10.1073/pnas.0608232104

van der Does, C., T. den Blaauwen, J.G. de Wit, E.H. Manting, N.A. Groot, P. Fekkes, and A.J. Driessen. 1996. SecA is an intrinsic subunit of the *Escherichia coli* preprotein translocase and exposes its carboxyl terminus to the periplasm. *Mol. Microbiol.* 22:619–629. http://dx.doi.org/10.1046/j.1365-2958.1996.d01-1712.x

Vassylyev, D.G., H. Mori, M.N. Vassylyeva, T. Tsukazaki, Y. Kimura, T.H. Tahirov, and K. Ito. 2006. Crystal structure of the translocation ATPase SecA from *Thermus thermophilus* reveals a parallel, head-to-head dimer. *J. Mol. Biol.* 364:248–258. http://dx.doi.org/10.1016/j.jmb.2006.09.061

Zimmer, J., and T.A. Rapoport. 2009. Conformational flexibility and peptide interaction of the translocation ATPase SecA. *J. Mol. Biol.* 394:606–612. http://dx.doi.org/10.1016/j.jmb.2009.10.024

Table S7. **Primers used in this study**

Primer identity	Forward/ reverse	Gene used for/mutation inserted	Restriction site	Sequence (5′–3′)
X107	Reverse	*secA 9-834*	BamHI	CGCGGATCCTTAAGGCATACGTACCTGAACTTTG
X131	Reverse	*secA*	BamHI	CGGCAGGGATCCTTATTGCAGGCGGCCATGGC
X178	Forward	*secA*	NdeI	GGCCCGTACATATGGTTTTCGGTAGTCGTAAC
X272	Forward	*secA 9-834*	KpnI	CGGGGTACCTTCGGTAGTCGTAACGATCGCACC
X434	Forward	*secA S809C*		CAAACGTGAATCGTTCTGCATGTTTGCAGCGATGC
X435	Reverse	*secA S809C*		GCATCGCTGCAAACATGCAGAACGATTCACGTTTG
X442	Forward	*secA P301C*		GAGTCTCTGTACTCTTGCGCCAACATCATGCTG
X443	Reverse	*secA P301C*		CAGCATGATGTTGGCGCAAGAGTACAGAGACC
X534	Forward	*secA C98A*		GTTCTTAACGAACGCGCCATCGCCGAAATGCGT
X535	Reverse	*secA C98A*		ACGCATTTCGGCGATGGCGCGTTCGTTAAGAAC
X560	Forward	*ProPhoA*	NdeI	GGGAATTCCATATGAAACAAAGCACTATTGCA
X561	Reverse	*ProPhoA*	XhoI	GACCCGCTCGAGTTTCAGCCCCAGAGCGGC
X646	Forward	*phoA*	NdeI	GGGAATTCCATATGACCCCAGAAATGCCTGTT
X649	Forward	*secA Q830C*		ACGCTGAGCAAAGTTTGCGTACGTATGCCTGAA
X650	Reverse	*secA Q830C*		TTCAGGCATACGTACGCAAACTTTGCTCAGCGT
X678	Forward	*phoA C194A*		GTGACCTCGCGCAAAGCCTACGGTCCGAGCGCG
X679	Reverse	*phoA C194A*		CGCGCTCGGACCGTAGGCTTTGCGCGAGGTCAC
X680	Forward	*phoA C204A*		GCGACCAGTGAAAAAGCTCCGGGTAACGCTCTG
X681	Reverse	*phoA C204A*		CAGAGCGTTACCCGGAGCTTTTTCACTGGTCGC
X682	Forward	*phoA C314A*		AAGCCCGCAGTCACCGCTACGCCAAATCCGCAA
X683	Reverse	*phoA C314A*		TTGCGGATTTGGCGTAGCGGTGACTGCGGGCTT
X684	Forward	*phoA C365A*		CATGCTGCGAATCCTGCTGGGCAAATTGGCGAG
X685	Reverse	*phoA C365A*		CTCGCCAATTTGCCCAGCAGGATTCGCAGCATG
X706	Forward	*secA M191C*		TACCTGCGCGACAACTGCGCGTTCAGCCCTGAA
X707	Reverse	*secA M191C*		TTCAGGGCTGAACGCGCAGTTGTCGCGCAGGTA
X722	Forward	*secA R850C*		CGTATGGAAGCCGAGTGCTTAGCGCAAATGCAG
X723	Reverse	*secA R850C*		CTGCATTTGCGCTAAGCACTCGGCTTCCATACG
X728	Reverse	*phoA*; anneals at K62	XhoI	GACCCGCTCGAGATATTTATCGCTAAGAGAATCACG
X729	Reverse	*phoA*; anneals at A82	XhoI	GACCCGCTCGAGATAGGCAGTAATTTCCGAGTC
X732	Forward	*proBglX*	NdeI	GGGAATTCCATATGAAATGGCTATGTTCAGTAGGAATCGCG
X734	Forward	*BglX*	NdeI	GGGAATTCCATATGGATGATTTATTCGGCAACCATCCATTAACG
X741	Forward	*ProPpiA*	NdeI	GGGAATTCCATATGTTCAAATCGACCCTGGCGGCG
X743	Forward	*PpiA*	NdeI	GGGAATTCCATATGGCAGCGAAAGGGGACCCG
X744	Forward	*proAmy1*	NdeI	GGGAATTCCATATGAAACTCGCCGCCTGTTTTCTGACA
X746	Forward	*Amy1*	NdeI	GGGAATTCCATATGGCCAGCTGGACTTCTCCGGG
X756	Forward	*ProOsmB*	NdeI	GGGAATTCCATATGTTTGTAACGAGCAAAAAAATGACCGCGG
X757	Reverse	*ProOsmB*	XhoI	GACCCGCTCGAGTTTACCGACCTGGTGACCAATAACACCT
X758	Forward	*OsmB*	NdeI	GGGAATTCCATATGTGTTCTAACTGGTCTAAACGGGACCG
X771	Forward	*ProYehR*	NdeI	GGGAATTCCATATGAAGGCTTTCAATAAGCTGTTTTCCCTCG
X772	Reverse	*ProYehR*	XhoI	GACCCGCTCGAGTTTCACTTCTTTAAAACCAGCGGCTTTCATCAC
X773	Forward	*YehR*	NdeI	GGGAATTCCATATGTGCGGTGACAAAGAAGAATCGAAGAAATTCAG
X806	Forward	*phoA*	NdeI, HindIII	GGGAATTCCATATGAAGCTTACACCAGAAATGCCTGTTCTGGAA
X807	Reverse	*phoA SP*	HindIII	CCCAAGCTTCCGGGCTTTTGTCACAGG
X926	Reverse	*proPhoA (1-30)*	XhoI	GACCCGCTCGAGATATTCCAGAACAGGCATTTCTGG
X927	Reverse	*proPhoA (1-40)*	XhoI	GACCCGCTCGAGATATGCAGTAATATCGCCCTGAGC
X928	Reverse	*proPhoA (1-50)*	XhoI	GACCCGCTCGAGATAATCACCCGTTAAACGGCGAGC
X936	Reverse	*phoA*; anneals at T122	XhoI	GACCCGCTCGAGTTAATAGGTGACGTAGTCCGGTTTG
X994	Reverse	*proPpiA (1-125)*	XhoI	GACCCGCTCGAGGCTGGTGGCGCTGTCTTTGTCAG
X993	Reverse	*proBglX (1-132)*	XhoI	GACCCGCTCGAGGAGGTTAAAAGACGAGGCCAGACCG
X995	Reverse	*proAmyI (1-131)*	XhoI	GACCCGCTCGAGCACTGTGAGCGGTAATCCATCCCATTTC
X998	Forward	*proPhoA (Δ21-121)*	HindIII	CCCAAGCTTACCGACTCGGCTGCATCAGCAACCG
X999	Forward	*phoA*; anneals at T123	NdeI	GGGAATTCCATATGACCGACTCGGCTGCATCAGCAACCG
X1032	Forward	*secA K268C*		TTCTCGGTGGACGAATGCTCTCGCCAGGTGAAC
X1033	Reverse	*secA K268C*		GTTCACCTGGCGAGAGCATTCGTCCACCGAGAA
X1040	Forward	*secA I597C*		GATGCGCTGATGCGTTGCTTTGCTTCCGACCGA
X1041	Reverse	*secA I597C*		TCGGTCGGAAGCAAAGCAACGCATCAGCGCATC

X1048	Forward	*proOsmB C24A*		ATGTCTCTGAGTGCCGCTTCTAACTGGTCTAAA
X1049	Reverse	*proOsmB C24A*		TTTAGACCAGTTAGAAGCGGCACTCAGAGACAT
X1058	Forward	*phoA I67A-I68T-L69A*		GATAAACCTGCAAAAAATGCTACTGCGCTGATTGGCGATGGGATG
X1059	Reverse	*phoA I67A-I68T-L69A*		CATCCCATCGCCAATCAGCGCAGTAGCATTTTTTGCAGGTTTATC
X1060	Forward	*phoA L70T-I71A on phoA I67A-I68T-L69A*		GCAAAAAATGCTACTGCGACGGCTGGCGATGGGATGGGGGAC
X1061	Reverse	*phoA L70T-I71A on phoA I67A-I68T-L69A*		GTCCCCCATCCCATCGCCAGCCGTCGCAGTAGCATTTTTTGC
X1062	Forward	*phoA F93A-F94A*		GCCGAAGGTGCGGGCGGCGCTGCTAAAGGTATAGATGCCTTA
X1063	Reverse	*phoA F93A-F94A*		TAAGGCATCTATACCTTTAGCAGCGCCGCCCGCACCTTCGGC
X1064	Forward	*phoA I97A on phoAF93A-F94A*		GGCGCTGCTAAAGGTGCAGATGCCTTACCGCTT
X1065	Reverse	*phoA I97A on phoAF93A-F94A*		AAGCGGTAAGGCATCTGCACCTTTAGCAGCGCC
X1066	Forward	*phoA L100A-L102A on phoA F93A-F94A-I97A*		GCTAAAGGTGCAGATGCCGCACCGGCTACCGGGCAATACACTCAC
X1067	Reverse	*phoA L100A-L102A on phoA F93A-F94A-I97A*		GTGAGTGTATTGCCCGGTAGCCGGTGCGGCATCTGCACCTTTAGC
X1068	Forward	*proPhoA (Δ23-249)*	HindIII	CCCAAGCTTCAGGCACAGGCGCGTG
X1069	Forward	*proPhoA (Δ23-349)*	HindIII	CCCAAGCTTAAACAGGATCATGCTGCGAATCCTT
X1075	Forward	*proLpp C21A*		ACTCTGCTGGCAGGTGCCTCCAGCAACGCTAAA
X1076	Reverse	*proLpp C21A*		TTTAGCGTTGCTGGAGGCACCTGCCAGCAGAGT
X1077	Forward	*proOsmB C24A*	NdeI	GGGAATTCCATATGGCTTCTAACTGGTCTAAACGGGACCGC
X1081	Forward	*ProLpp*	NdeI	GGGAATTCCATATGAAAGCTACTAAACTGGTACTGGGCG
X1082	Reverse	*ProLpp*	XhoI	GACCCGCTCGAGCATCTTGCGGTATTTAGTAGCCATGTTGTCCAG
X1128	Forward	*ProSpy*	NdeI	GGGAATTCCATATGCGTAAATTAACTGCACTGTTTGTTGCCTC
X1129	Reverse	*ProSpy*	XhoI	GACCCGCTCGAGCATTTCAGCAGTTGCAGGCATTTTACCTTTTGC
X1130	Forward	*Spy*	NdeI	GGGAATTCCATATGGCAGACACCACTACCGCAGCAC
X1146	Reverse	*phoA 1-122*	XhoI	GACCCGCTCGAGGGTGACGTAGTCCGGTTTGCC
X1171	Forward	*Lpp C21A-I29A*	NdeI	GGGAATTCCATATGGCCTCCAGCAACGCTAAAGCCGATCAG
X1184	Forward	*phoA (250-471)*	NdeI	GGGAATTCCATATGCAGGCACAGGCGCGTG
X1185	Forward	*phoA (350-471)*	NdeI	GGGAATTCCATATGAAACAGGATCATGCTGCGAATCC
X1188	Forward	*proLpp I27A-L30A*		TCCAGCAACGCTAAAGCCGATCAGGCGTCTTCT
X1189	Reverse	*proLpp I27A-L30A*		AGAAGACGCCTGATCGGCTTTAGCGTTGCTGGA
X1190	Forward	*proLpp I27A-L30A-V34A*		GCTAAAGCCGATCAGGCGTCTTCTGACGCTCAG
X1191	Reverse	*proLpp I27A-L30A-V34A*		CTGAGCGTCAGAAGACGCCTGATCGGCTTTAGC
X1192	Forward	*proLpp L30A-V34A-L37A*		CAGGCGTCTTCTGACGCTCAGACTGCGAACGCT
X1193	Reverse	*proLpp L30A-V34A-L37A*		AGCGTTCGCAGTCTGAGCGTCAGAAGACGCCTG
X1194	Forward	*proLpp V34A-L37A-V41A*		TCTGACGCTCAGACTGCGAACGCTAAAGCTGAC
X1195	Reverse	*proLpp V34A-L37A-V41A*		GTCAGCTTTAGCGTTCGCAGTCTGAGCGTCAGA
X1196	Forward	*proLpp L37A-V41A-L44A*		ACTGCGAACGCTAAAGCTGACCAGGCGAGCAAC
X1197	Reverse	*proLpp L37A-V41A-L44A*		GTTGCTCGCCTGGTCAGCTTTAGCGTTCGCAGT
X1198	Forward	*proLpp V41A-L44A-V48A*		GCTAAAGCTGACCAGGCGAGCAACGACGCGAAC
X1199	Reverse	*proLpp V41A-L44A-V48A*		GTTCGCGTCGTTGCTCGCCTGGTCAGCTTTAGC
X1200	Forward	*proLpp L44A-V48A*		CAGGCGAGCAACGACGCGAACGCAATGCGTTCC
X1201	Reverse	*proLpp L44A-V48A*		GGAACGCATTGCGTTCGCGTCGTTGCTCGCCTG
X1202	Forward	*proLpp V55A*		GCAATGCGTTCCGACGCTCAGGCTGCTAAAGAT
X1203	Reverse	*proLpp V55A*		ATCTTTAGCAGCCTGAGCGTCGGAACGCATTGC
X1204	Forward	*proLpp L69A*		CGTGCTAACCAGCGTGCGGACAACATGGCTACT
X1205	Reverse	*proLpp L69A*		AGTAGCCATGTTGTCCGCACGCTGGTTAGCACG
X1393	Forward	*ProHdeN*	NdeI	GGGAATTCCATATGAAAAAAGTATTAGGCGTTATTCTTG
X1394	Reverse	*ProHdeN*	XhoI	GACCCGCTCGAGCATATCTTTCTTAATTTTGTCCC
X1395	Forward	*HdeN*	NdeI	GGGAATTCCATATGGCGGATGCGCAAAAAGCAGCTGAT
X1422	Forward	*ProYncJ*	NdeI	GGGAATTCCATATGTTTACGAAGGCGTTATCGGTTG
X1423	Reverse	*ProYncJ*	XhoI	GACCCGCTCGAGACGTTGTCCCGCTTCTGGTTTG
X1424	Forward	*YncJ*	NdeI	GGGAATTCCATATGGGGCACAAAGGACATGAATTT
X1570	Reverse	*YncJ*; inserts 2 Methionine at the end of the genes	XhoI	GACCCGCTCGAGCATCATACGTTGTCCCGCTTCTGG
X1571	Reverse	*HdeN*; inserts 2 Methionine at the end of the genes	XhoI	GACCCGCTCGAGCATCATATCTTTCTTAATTTTGTCCC

X1572	Reverse	*phoA*; inserts 1 Methionine at the end of the genes	XhoI	GACCCGCTCGAGcatGGTCCCGGTGGTGGTTTTC
X1915	Forward	Destroys HindIII site for constructing pIMBB1570		CACCACCACCACTGAAAACTTGGCTGTTTTGGC
X1916	Reverse	Destroys HindIII site for constructing pIMBB1570		GCCAAAACAGCCAAGTTTTCAGTGGTGGTGGTG
X1926	Forward	*Gentamicin gene*	MscI	GACCCGTGGCCAGCCTCGACTTCCCTGCTGCC
X1927	Reverse	*Gentamicin gene*	ScaI	AAATTTAGTACTCCAAGGGCATGGTAAAG
X1954	Forward	*secA I224A-I225A*		GAAGCGCGTACACCGCTGGCGGCGTCCGGCCCGGCAGAAGAC
X1955	Reverse	*secA I224A-I225A*		GTCTTCTGCCGGGCCGGACGCCGCCAGCGGTGTACGCGCTTC
X1958	Forward	*secA M191A-F193A*		GACTACCTGCGCGACAACGCGGCGGCGAGCCCTGAAGAACGTGTA
X1959	Reverse	*secA M191A-F193A*		TACACGTTCTTCAGGGCTCGCCGCCGCGTTGTCGCGCAGGTAGTC
X1973	Reverse	*ProYncJ*	HindIII	CCCAAGCTTACGTTGTCCCGCTTCTGGTTTG

The following primers either from the microchemistry facility at the Institute of Molecular Biology and Biotechnology, Macrogen, or Metabion were used for plasmid constructs (as indicated).

Table S8. **Predicted hydrodynamic diameters of preproteins handled by the Sec translocase**

Entry name (Uniprot)	Length (amino acids)	Folded D_H (nm)	Unfolded D_H (nm)
YEEJ_ECOLI	2358	9.14	37.41
YDBA_ECOLI	2003	8.71	34.07
YFHM_ECOLI	1653	8.24	30.52
YFAS_ECOLI	1534	8.06	29.24
YPJA_ECOLI	1526	8.05	29.15
ACFD_ECOLI	1520	8.04	29.09
YDEK_ECOLI	1325	7.72	26.89
YHDP_ECOLI	1266	7.62	26.20
YFAL_ECOLI	1250	7.59	26.01
BCSC_ECOLI	1157	7.42	24.88
AG43_ECOLI	1039	7.19	23.39
NFRA_ECOLI	990	7.09	22.75
YAIT_ECOLI	968	7.04	22.46
PTRA_ECOLI	962	7.03	22.38
PQQL_ECOLI	931	6.96	21.97
CHIA_ECOLI	897	6.89	21.50
YFCU_ECOLI	881	6.85	21.28
FIMD_ECOLI	878	6.84	21.24
SFMD_ECOLI	867	6.82	21.09
YCBS_ECOLI	866	6.82	21.08
HTRE_ECOLI	865	6.81	21.06
YEJO_ECOLI	863	6.81	21.03
YAGX_ECOLI	841	6.76	20.72
YRAJ_ECOLI	838	6.75	20.68
YEHB_ECOLI	826	6.72	20.51
YQIG_ECOLI	821	6.71	20.44
YBGQ_ECOLI	815	6.70	20.36
DMSA_ECOLI	814	6.69	20.34
YAET_ECOLI	810	6.68	20.28
TORZ_ECOLI	809	6.68	20.27
PGAA_ECOLI	807	6.68	20.24
YNFF_ECOLI	807	6.68	20.24
YHCD_ECOLI	793	6.64	20.04
YDDB_ECOLI	790	6.64	20.00
LPTD_ECOLI	784	6.62	19.91
YGJK_ECOLI	783	6.62	19.89
FECA_ECOLI	774	6.60	19.76
YDBD_ECOLI	768	6.58	19.67
BGLX_ECOLI	765	6.57	19.63
FIU_ECOLI	760	6.56	19.56
AMO_ECOLI	757	6.55	19.51
FHUA_ECOLI	747	6.53	19.36
FEPA_ECOLI	746	6.53	19.35
FHUE_ECOLI	729	6.48	19.10
YNCD_ECOLI	700	6.40	18.66
GFCD_ECOLI	698	6.40	18.63
YJBH_ECOLI	698	6.40	18.63
PRC_ECOLI	682	6.36	18.38
YRAM_ECOLI	678	6.35	18.32
AMY1_ECOLI	676	6.34	18.29
PGAB_ECOLI	672	6.33	18.23
CIRA_ECOLI	663	6.30	18.09
YJCS_ECOLI	661	6.30	18.05
GSPD_ECOLI	650	6.27	17.88
CPDB_ECOLI	647	6.26	17.83
SLT_ECOLI	645	6.25	17.80
YACH_ECOLI	617	6.17	17.36
BTUB_ECOLI	614	6.16	17.31

Entry name (Uniprot)	Length (amino acids)	Folded D_H (nm)	PDB code	Polypeptide chain analyzed
ACRA_ECOLI	268	8.04	2F1M	A
AGP_ECOLI	391	5.58	1NT4	A
ALSB_ECOLI	288	7.56	1GUD	A
AMIC_ECOLI	383	5.7	4BIN	A
AMID_ECOLI	259	5.38	2WKX	A
AMO_ECOLI	721	7.22	1QAF	A
AMPC_ECOLI	358	5.08	2R9W	A
APBE_ECOLI	331	5.22	2O18	A
APHA_ECOLI	211	4.7	2B82	A
ARAF_ECOLI	306	5.52	2WRZ	A
ASPG2_ECOLI	326	5.26	1JJA	A
BAMA_ECOLI	390	9.28	3EFC	A
BAMB_ECOLI	371	5.28	2YH3	A
BAMC_ECOLI	119	3.48	2YH5	A
BAMD_ECOLI	223	6.14	3Q5M	A
BAME_ECOLI	93	5.48	2KXX	A
BLC_ECOLI	155	3.88	2ACO	A
BTUB_ECOLI	590	6.54	2GSK	A
BTUF_ECOLI	244	5.26	1N4D	A
CIRA_ECOLI	638	6.32	2HDF	A
CPXP_ECOLI	130	5	3ITF	A
CUEO_ECOLI	488	5.38	3PAU	A
CUSB_ECOLI	330	9.4	3T51	B
CUSC_ECOLI	440	6.98	4K34	A
CUSF_ECOLI	88	2.98	2VB2	A
DACA_ECOLI	363	6.08	1Z6F	A
DACB_ECOLI	457	6.48	2EX8	A
DACC_ECOLI	351	5.96	3ITA	A
DEGP_ECOLI	448	6.28	3MH6	A
DEGQ_ECOLI	427	4.14	3STI	A
DEGS_ECOLI	329	6.04	3GCN	A
DGAL_ECOLI	309	5.3	2HPH	A
DPPA_ECOLI	507	4.4	1DPE	A
DSBA_ECOLI	189	4.4	1A2J	A
DSBC_ECOLI	217	4.9	1JZD	A
DSBG_ECOLI	231	5.96	2H0G	A
ECOT_ECOLI	142	5.42	1ECY	A
EFEB_ECOLI	388	5.52	2Y4E	A
EMTA_ECOLI	187	4.12	4HJV	A
ENVC_ECOLI	142	6.26	4BH5	A
FADL_ECOLI	421	6.44	3PGR	A
FDNG_ECOLI	1015	7.16	1KQF	A
FECA_ECOLI	741	6.44	1PO0	A
FEPA_ECOLI	724	6.48	1FEP	A
FEPB_ECOLI	318	5.18	3TLK	A
FHUA_ECOLI	714	6.62	1QFF	A
FHUD_ECOLI	266	4.94	1ESZ	A
FIMC_ECOLI	205	6.82	3BWU	C
FIMD_ECOLI	125	4.22	3BWU	D
FKBA_ECOLI	224	6.82	1Q6U	A
FTSP_ECOLI	443	5.32	2UXT	A
GFCB_ECOLI	198	4.48	2IN5	A
GGT_ECOLI	366	6.24	2E0X	A
GLNH_ECOLI	226	5.02	1GGG	A
GLPQ_ECOLI	334	5.02	1T8Q	A
GSIB_ECOLI	489	6.2	1UQW	A
GSPH_ECOLI	140	4.52	2KNQ	A
GUN_ECOLI	347	4.96	3QXF	A

YEJA_ECOLI	604	6.13	17.15
TRAN_ECOLI	602	6.13	17.11
GGT_ECOLI	580	6.06	16.75
YTFM_ECOLI	577	6.05	16.70
YFBK_ECOLI	575	6.05	16.67
TREA_ECOLI	565	6.02	16.50
YFAA_ECOLI	562	6.01	16.45
YDEN_ECOLI	560	6.00	16.42
ASLA_ECOLI	551	5.97	16.27
OPGD_ECOLI	551	5.97	16.27
USHA_ECOLI	550	5.97	16.25
YFAQ_ECOLI	549	5.97	16.23
YAGW_ECOLI	547	5.96	16.20
SAPA_ECOLI	547	5.96	16.20
OPPA_ECOLI	543	5.95	16.13
BGLH_ECOLI	538	5.93	16.05
MPPA_ECOLI	537	5.93	16.03
DPPA_ECOLI	535	5.92	16.00
YGIS_ECOLI	535	5.92	16.00
NIKA_ECOLI	524	5.88	15.81
MLTF_ECOLI	518	5.86	15.70
CUEO_ECOLI	516	5.86	15.67
DDPA_ECOLI	516	5.86	15.67
GSIB_ECOLI	512	5.84	15.60
OPGG_ECOLI	511	5.84	15.58
YDGA_ECOLI	502	5.81	15.42
YHJJ_ECOLI	498	5.80	15.35
TOLC_ECOLI	493	5.78	15.26
MDTP_ECOLI	488	5.76	15.18
YFGC_ECOLI	487	5.76	15.16
MDTQ_ECOLI	478	5.73	15.00
NRFA_ECOLI	478	5.73	15.00
DACB_ECOLI	477	5.73	14.98
DEGP_ECOLI	474	5.71	14.92
PPB_ECOLI	471	5.70	14.87
SUFI_ECOLI	470	5.70	14.85
YBFM_ECOLI	468	5.69	14.82
YHJA_ECOLI	465	5.68	14.76
YCHO_ECOLI	464	5.68	14.74
YAHJ_ECOLI	460	5.66	14.67
PAT_ECOLI	459	5.66	14.65
TRAH1_ECOLI	458	5.66	14.63
CUSC_ECOLI	457	5.65	14.62
DEGQ_ECOLI	455	5.65	14.58
MLTD_ECOLI	452	5.64	14.52
FADL_ECOLI	446	5.61	14.41
LAMB_ECOLI	446	5.61	14.41
AMIB_ECOLI	445	5.61	14.39
YDDW_ECOLI	439	5.59	14.28
UGPB_ECOLI	438	5.58	14.26
YNJE_ECOLI	435	5.57	14.21
YFEW_ECOLI	434	5.57	14.19
PPA_ECOLI	432	5.56	14.15
YCJN_ECOLI	430	5.55	14.12
TOLB_ECOLI	430	5.55	14.12
SURA_ECOLI	428	5.55	14.08
YBHC_ECOLI	427	5.54	14.06
YCDB_ECOLI	423	5.53	13.98
UIDC_ECOLI	421	5.52	13.95
WECC_ECOLI	420	5.52	13.93
YIBP_ECOLI	419	5.51	13.91
AMIC_ECOLI	417	5.50	13.87
MDTA_ECOLI	415	5.50	13.83
AGP_ECOLI	413	5.49	13.79

HDEA_ECOLI	89	3.24	1DJ8	A
HDEB_ECOLI	79	3.16	2XUV	A
HISJ_ECOLI	233	4.62	1HSL	A
HIUH_ECOLI	114	3.76	2G2P	A
HSLJ_ECOLI	116	3.96	2KTS	A
IVY_ECOLI	128	3.68	1GPQ	A
LAMB_ECOLI	421	5.86	1MPM	A
LIVJ_ECOLI	344	5.14	1Z17	A
LIVK_ECOLI	346	5.34	1USI	A
LOLA_ECOLI	182	4.16	2ZPC	A
LOLB_ECOLI	186	4.08	1IWM	A
LPP_ECOLI	56	6.2	1EQ7	A
LPTA_ECOLI	159	3.94	2R19	A
MALE_ECOLI	358	6.36	3IOW	A
MATB_ECOLI	155	4.8	3QS3	A
MEPA_ECOLI	255	4.56	1U10	A
MLIC_ECOLI	82	3.64	2F09	A
MLTA_ECOLI	344	5.72	2GAE	A
MLTB_ECOLI	320	5.64	1LTM	A
MLTD_ECOLI	48	3.02	1E0G	A
MODA_ECOLI	233	4.68	1WOD	A
MPPA_ECOLI	515	6.06	3O9P	A
NANC_ECOLI	215	4.76	2WJQ	A
NANM_ECOLI	349	5.04	2UVK	A
NAPA_ECOLI	792	6.48	2NYA	A
NIKA_ECOLI	502	6.38	2NOO	A
NLPE_ECOLI	216	8.02	2Z4H	A
NLPI_ECOLI	275	4.92	1XNF	A
NRFA_ECOLI	441	5.86	2RF7	A
NRFB_ECOLI	163	4.18	2OZY	A
OMPA_ECOLI	171	4.26	1QJP	A
OMPC_ECOLI	346	5.5	2J1N	A
OMPF_ECOLI	362	5.66	3HWB	A
OMPG_ECOLI	280	5.3	2IWW	A
OMPT_ECOLI	297	5.98	1I78	A
OMPW_ECOLI	191	4.94	2F1V	A
OMPX_ECOLI	148	4.76	1QJ8	A
OPGG_ECOLI	489	6.38	1TXK	A
OPPA_ECOLI	516	6.34	3TCH	A
PA1_ECOLI	269	5	1ILZ	A
PAGP_ECOLI	161	6.96	1MM5	A
PAL_ECOLI	109	3.56	2HQS	C
PANE_ECOLI	303	4.96	1YJQ	A
PGAB_ECOLI	614	6.86	4F9D	A
PHNP_ECOLI	252	4.64	3G1P	A
PHOE_ECOLI	330	5.62	1PHO	A
PLIG_ECOLI	111	3.74	4DY3	A
POTD_ECOLI	325	5.12	1POT	A
POTF_ECOLI	344	5.28	4JDF	A
PPA_ECOLI	410	5.6	1DKN	A
PPB_ECOLI	449	5.68	1KH5	A
PPIA_ECOLI	166	3.76	1J2A	A
PROX_ECOLI	309	5.48	1R9L	A
PSPE_ECOLI	85	3.5	2JTR	A
PSTS_ECOLI	321	5.18	1A40	A
PTFB1_ECOLI	108	3.74	2KYR	A
PTRA_ECOLI	939	8.76	1Q2L	A
RBSB_ECOLI	271	5.14	1DRK	A
RCSF_ECOLI	118	7.38	2L8Y	A
RMLA1_ECOLI	293	4.84	1H5S	A
RNI_ECOLI	245	4.4	2PQX	A
RSEB_ECOLI	296	5.4	2V42	A
SKP_ECOLI	142	6.48	1SG2	A
SLT_ECOLI	618	7.36	1QTE	A

INTA_ECOLI	413	5.49	13.79
HOFQ_ECOLI	412	5.49	13.77
YADC_ECOLI	412	5.49	13.77
YADE_ECOLI	409	5.47	13.72
CUSB_ECOLI	407	5.47	13.68
DACA_ECOLI	403	5.45	13.60
DACC_ECOLI	400	5.44	13.54
YEDS_ECOLI	397	5.43	13.48
ACRA_ECOLI	397	5.43	13.48
MALE_ECOLI	396	5.42	13.47
YFGL_ECOLI	392	5.41	13.39
YCIM_ECOLI	389	5.39	13.33
YIEL_ECOLI	389	5.39	13.33
YNJB_ECOLI	388	5.39	13.31
DACD_ECOLI	388	5.39	13.31
EMRK_ECOLI	387	5.39	13.29
AMPH_ECOLI	385	5.38	13.25
ACRE_ECOLI	385	5.38	13.25
MDTE_ECOLI	385	5.38	13.25
YDCS_ECOLI	381	5.36	13.17
NLPD_ECOLI	379	5.35	13.13
WZA_ECOLI	379	5.35	13.13
GFCE_ECOLI	379	5.35	13.13
OMPN_ECOLI	377	5.34	13.09
AMPC_ECOLI	377	5.34	13.09
YCDO_ECOLI	375	5.34	13.05
MBHT_ECOLI	372	5.32	12.99
YLII_ECOLI	371	5.32	12.97
POTF_ECOLI	370	5.32	12.95
LIVK_ECOLI	369	5.31	12.93
GUN_ECOLI	368	5.31	12.91
NANM_ECOLI	368	5.31	12.91
OMPC_ECOLI	367	5.30	12.89
LIVJ_ECOLI	367	5.30	12.89
MLTA_ECOLI	365	5.29	12.85
NMPC_ECOLI	365	5.29	12.85
YAIW_ECOLI	364	5.29	12.83
YRAK_ECOLI	363	5.29	12.81
RLPA_ECOLI	362	5.28	12.79
OMPF_ECOLI	362	5.28	12.79
MLTB_ECOLI	361	5.28	12.77
MLTC_ECOLI	359	5.27	12.73
GLPQ_ECOLI	358	5.26	12.71
YGJJ_ECOLI	356	5.26	12.67
YCBT_ECOLI	356	5.26	12.67
DEGS_ECOLI	355	5.25	12.65
YQII_ECOLI	354	5.25	12.63
YBGO_ECOLI	353	5.24	12.61
YNCE_ECOLI	353	5.24	12.61
PHOE_ECOLI	351	5.23	12.57
APBE_ECOLI	351	5.23	12.57
YIIG_ECOLI	351	5.23	12.57
POTD_ECOLI	348	5.22	12.51
ASPG2_ECOLI	348	5.22	12.51
YPFG_ECOLI	347	5.22	12.48
OMPA_ECOLI	346	5.21	12.46
PSTS_ECOLI	346	5.21	12.46
IAP_ECOLI	345	5.21	12.44
NLPB_ECOLI	344	5.20	12.42
YEHA_ECOLI	344	5.20	12.42
TORT_ECOLI	342	5.19	12.38
YHDW_ECOLI	341	5.19	12.36
LSRB_ECOLI	340	5.19	12.34
PHND_ECOLI	338	5.18	12.30

SODC_ECOLI	154	3.84	1ESO	A
SODF_ECOLI	192	4.14	1ISC	A
SODM_ECOLI	205	4.32	1IX9	A
SPR_ECOLI	126	4.32	2K1G	A
SSUA_ECOLI	295	6.16	2X26	A
SURA_ECOLI	103	3.46	2PV1	A
TAMA_ECOLI	254	7.46	4BZA	A
TESA_ECOLI	182	4.02	1JRL	A
THIB_ECOLI	309	4.94	2QRY	A
TOLB_ECOLI	408	5.68	2HQS	A
TOLC_ECOLI	450	10.42	2VDD	A
TREA_ECOLI	535	5.92	2JG0	A
TrxB_ECOLI	316	5.74	1TDE	A
TSX_ECOLI	272	5.22	1TLY	A
UGPB_ECOLI	415	5.42	4AQ4	A
USHA_ECOLI	525	6.46	1OI8	A
VISC_ECOLI	365	5.66	4K22	A
XYLF_ECOLI	307	5.44	3M9X	A
YAJI_ECOLI	159	8.32	2JWY	A
YBCL_ECOLI	162	3.92	1FUX	A
YBGF_ECOLI	75	7.48	2XDJ	A
YBHC_ECOLI	399	5.32	3GRH	A
YCEB_ECOLI	167	4.82	3L6I	A
YCEI_ECOLI	191	4.36	1Y0G	A
YEDY_ECOLI	290	4.46	1XDQ	A
YEHR_ECOLI	130	5	2JOE	A
YFEY_ECOLI	164	4.18	2QZB	A
YGDR_ECOLI	51	3.26	2JN0	A
YGIW_ECOLI	109	3.28	1NNX	A
YGJK_ECOLI	760	7.26	3W7T	A
YIAD_ECOLI	141	4.86	2K1S	A
YLII_ECOLI	350	5.08	2G8S	A
YMGD_ECOLI	90	3.34	2LRM	A
YNCE_ECOLI	353	4.7	3VGZ	A
YNJE_ECOLI	412	5.72	2WLX	A
YODA_ECOLI	193	4.38	1OEK	A
YTFQ_ECOLI	297	5.16	2VK2	A
ZNUA_ECOLI	284	4.82	2PRS	A

CYSP_ECOLI	338	5.18	12.30
YGGM_ECOLI	335	5.16	12.24
YNHG_ECOLI	334	5.16	12.21
YEDY_ECOLI	334	5.16	12.21
DGAL_ECOLI	332	5.15	12.17
PROX_ECOLI	330	5.14	12.13
XYLF_ECOLI	330	5.14	12.13
TRAU_ECOLI	330	5.14	12.13
SUBI_ECOLI	329	5.14	12.11
ARAF_ECOLI	329	5.14	12.11
YIAO_ECOLI	328	5.13	12.09
HYBA_ECOLI	328	5.13	12.09
THIB_ECOLI	327	5.13	12.07
GSPK_ECOLI	327	5.13	12.07
SFMH_ECOLI	327	5.13	12.07
YPHF_ECOLI	327	5.13	12.07
YDJG_ECOLI	326	5.12	12.05
TRXB_ECOLI	321	5.10	11.94
YCFS_ECOLI	320	5.09	11.92
TAUA_ECOLI	320	5.09	11.92
YIBQ_ECOLI	319	5.09	11.90
SSUA_ECOLI	319	5.09	11.90
FEPB_ECOLI	318	5.09	11.88
RSEB_ECOLI	318	5.09	11.88
YTFQ_ECOLI	318	5.09	11.88
OMPT_ECOLI	317	5.08	11.85
YDGH_ECOLI	314	5.07	11.79
ALSB_ECOLI	311	5.05	11.73
PBP7_ECOLI	310	5.05	11.70
ZNUA_ECOLI	310	5.05	11.70
ERFK_ECOLI	310	5.05	11.70
YQHG_ECOLI	308	5.04	11.66
MALM_ECOLI	306	5.03	11.62
YBIS_ECOLI	306	5.03	11.62
OSMF_ECOLI	305	5.02	11.60
YDEQ_ECOLI	304	5.02	11.57
PANE_ECOLI	303	5.01	11.55
GLTI_ECOLI	302	5.01	11.53
OMPG_ECOLI	301	5.00	11.51
FECB_ECOLI	300	5.00	11.49
FIMH_ECOLI	300	5.00	11.49
RBSB_ECOLI	296	4.98	11.40
YBCH_ECOLI	296	4.98	11.40
FHUD_ECOLI	296	4.98	11.40
NLPI_ECOLI	294	4.97	11.35
TSX_ECOLI	294	4.97	11.35
PA1_ECOLI	289	4.95	11.24
AMIA_ECOLI	289	4.95	11.24
YGHF_ECOLI	288	4.94	11.22
YGEQ_ECOLI	278	4.89	11.00
CSGG_ECOLI	277	4.88	10.97
AMID_ECOLI	276	4.88	10.95
YAEF_ECOLI	274	4.87	10.91
YEEZ_ECOLI	274	4.87	10.91
MEPA_ECOLI	274	4.87	10.91
BAX_ECOLI	274	4.87	10.91
YDGD_ECOLI	273	4.86	10.88
YFCO_ECOLI	273	4.86	10.88
NLPA_ECOLI	272	4.86	10.86
YDHO_ECOLI	271	4.85	10.84
METQ_ECOLI	271	4.85	10.84
FKBA_ECOLI	270	4.85	10.81
RNI_ECOLI	268	4.84	10.77
FLIY_ECOLI	266	4.83	10.72

BTUF_ECOLI	266	4.83	10.72
YBGF_ECOLI	263	4.81	10.65
YAFT_ECOLI	261	4.80	10.61
HISJ_ECOLI	260	4.79	10.58
ARGT_ECOLI	260	4.79	10.58
YFAP_ECOLI	258	4.78	10.54
MODA_ECOLI	257	4.78	10.51
YAIO_ECOLI	257	4.78	10.51
YCAL_ECOLI	254	4.76	10.44
YFEN_ECOLI	254	4.76	10.44
YIGE_ECOLI	254	4.76	10.44
GLTF_ECOLI	254	4.76	10.44
YGGG_ECOLI	252	4.75	10.40
PHNP_ECOLI	252	4.75	10.40
YDIY_ECOLI	252	4.75	10.40
MLAA_ECOLI	251	4.75	10.37
YGER_ECOLI	251	4.75	10.37
YFCS_ECOLI	250	4.74	10.35
YQIH_ECOLI	249	4.73	10.33
YAFL_ECOLI	249	4.73	10.33
MIPA_ECOLI	248	4.73	10.30
GLNH_ECOLI	248	4.73	10.30
GFCC_ECOLI	248	4.73	10.30
DSBG_ECOLI	248	4.73	10.30
TRAF_ECOLI	247	4.72	10.28
YGGE_ECOLI	246	4.72	10.25
ECPD_ECOLI	246	4.72	10.25
YAFK_ECOLI	246	4.72	10.25
YIAT_ECOLI	246	4.72	10.25
YFIO_ECOLI	245	4.71	10.23
YJBG_ECOLI	245	4.71	10.23
TRAT1_ECOLI	244	4.71	10.21
ARTI_ECOLI	243	4.70	10.18
ARTJ_ECOLI	243	4.70	10.18
YBGP_ECOLI	242	4.70	10.16
TRAK1_ECOLI	242	4.70	10.16
FIMC_ECOLI	241	4.69	10.13
YGGN_ECOLI	239	4.68	10.09
YEHC_ECOLI	239	4.68	10.09
YHCF_ECOLI	238	4.67	10.06
NANC_ECOLI	238	4.67	10.06
YFHG_ECOLI	237	4.67	10.04
APHA_ECOLI	237	4.67	10.04
NLPE_ECOLI	236	4.66	10.01
DSBC_ECOLI	236	4.66	10.01
YCBF_ECOLI	236	4.66	10.01
YAGV_ECOLI	236	4.66	10.01
YIAF_ECOLI	236	4.66	10.01
YNFC_ECOLI	236	4.66	10.01
END1_ECOLI	235	4.65	9.99
YCBR_ECOLI	233	4.64	9.94
FLGH_ECOLI	232	4.64	9.92
YHJY_ECOLI	232	4.64	9.92
YJAH_ECOLI	231	4.63	9.89
YRAI_ECOLI	231	4.63	9.89
FLGD_ECOLI	231	4.63	9.89
OMPL_ECOLI	230	4.63	9.87
SFMC_ECOLI	230	4.63	9.87
YJCO_ECOLI	229	4.62	9.84
YDJY_ECOLI	225	4.60	9.74
YHCA_ECOLI	224	4.59	9.72
YDCL_ECOLI	222	4.58	9.67
MATC_ECOLI	222	4.58	9.67
YDHX_ECOLI	222	4.58	9.67

YCCT_ECOLI	220	4.57	9.62
YIAD_ECOLI	219	4.56	9.59
FLGA_ECOLI	219	4.56	9.59
YIDX_ECOLI	218	4.55	9.57
YODA_ECOLI	216	4.54	9.52
GFCB_ECOLI	214	4.53	9.47
YCFM_ECOLI	213	4.52	9.44
YJBF_ECOLI	212	4.52	9.42
OMPW_ECOLI	212	4.52	9.42
TRBC_ECOLI	212	4.52	9.42
MLAC_ECOLI	211	4.51	9.39
YFDX_ECOLI	211	4.51	9.39
TRAW_ECOLI	210	4.50	9.37
TESA_ECOLI	208	4.49	9.32
DSBA_ECOLI	208	4.49	9.32
LOLB_ECOLI	207	4.49	9.29
YFAT_ECOLI	207	4.49	9.29
RNFG_ECOLI	206	4.48	9.26
YIJF_ECOLI	205	4.47	9.24
EMTA_ECOLI	203	4.46	9.19
LOLA_ECOLI	203	4.46	9.19
YIIX_ECOLI	202	4.45	9.16
OSMY_ECOLI	201	4.45	9.13
YADL_ECOLI	201	4.45	9.13
AIS_ECOLI	200	4.44	9.11
YIIQ_ECOLI	199	4.43	9.08
NRFG_ECOLI	198	4.43	9.06
YADK_ECOLI	198	4.43	9.06
GSPJ_ECOLI	195	4.41	8.98
MATB_ECOLI	195	4.41	8.98
YADN_ECOLI	194	4.40	8.95
YRAH_ECOLI	194	4.40	8.95
LPTE_ECOLI	193	4.39	8.92
YEAY_ECOLI	193	4.39	8.92
YAJG_ECOLI	192	4.39	8.90
YRAP_ECOLI	191	4.38	8.87
YFEY_ECOLI	191	4.38	8.87
YCEI_ECOLI	191	4.38	8.87
YBAY_ECOLI	190	4.37	8.85
PPIA_ECOLI	190	4.37	8.85
YBFC_ECOLI	189	4.37	8.82
YADM_ECOLI	189	4.37	8.82
SPR_ECOLI	188	4.36	8.79
SLP_ECOLI	188	4.36	8.79
NRFB_ECOLI	188	4.36	8.79
YBGD_ECOLI	188	4.36	8.79
YFCV_ECOLI	187	4.35	8.76
YMBA_ECOLI	187	4.35	8.76
YCEB_ECOLI	186	4.35	8.74
CRCA_ECOLI	186	4.35	8.74
LPTA_ECOLI	185	4.34	8.71
DCRB_ECOLI	185	4.34	8.71
YBET_ECOLI	184	4.33	8.68
YTFJ_ECOLI	184	4.33	8.68
YGIL_ECOLI	183	4.33	8.66
MLAD_ECOLI	183	4.33	8.66
YBCL_ECOLI	183	4.33	8.66
FIMA1_ECOLI	182	4.32	8.63
YHCE_ECOLI	181	4.31	8.60
TRBB_ECOLI	181	4.31	8.60
YFAZ_ECOLI	180	4.31	8.58
SFMA_ECOLI	180	4.31	8.58
YEHD_ECOLI	180	4.31	8.58
YAJI_ECOLI	179	4.30	8.55

FIMI_ECOLI	179	4.30	8.55
YCBQ_ECOLI	179	4.30	8.55
YFCP_ECOLI	179	4.30	8.55
YFGI_ECOLI	179	4.30	8.55
BLC_ECOLI	177	4.29	8.49
LYSQ_ECOLI	177	4.29	8.49
FIMF_ECOLI	176	4.28	8.47
YDES_ECOLI	176	4.28	8.47
PAL_ECOLI	173	4.26	8.38
SODC_ECOLI	173	4.26	8.38
YFGH_ECOLI	172	4.25	8.36
YFIR_ECOLI	172	4.25	8.36
YBJP_ECOLI	171	4.24	8.33
TRAV_ECOLI	171	4.24	8.33
OMPX_ECOLI	171	4.24	8.33
SFMF_ECOLI	171	4.24	8.33
YCBV_ECOLI	171	4.24	8.33
SECM_ECOLI	170	4.23	8.30
YFCR_ECOLI	170	4.23	8.30
GSPH_ECOLI	169	4.23	8.27
X19F_ECOLI	169	4.23	8.27
YOEA_ECOLI	167	4.21	8.22
YDER_ECOLI	167	4.21	8.22
FIMG_ECOLI	167	4.21	8.22
CPXP_ECOLI	166	4.21	8.19
RZPQ_ECOLI	165	4.20	8.16
LYSD_ECOLI	165	4.20	8.16
YBFP_ECOLI	164	4.19	8.13
YJJA_ECOLI	164	4.19	8.13
ECOT_ECOLI	162	4.18	8.07
YECT_ECOLI	162	4.18	8.07
YFCQ_ECOLI	162	4.18	8.07
SKP_ECOLI	161	4.17	8.05
SPY_ECOLI	161	4.17	8.05
YFIB_ECOLI	160	4.16	8.02
PBL_ECOLI	158	4.15	7.96
IVY_ECOLI	157	4.14	7.93
CREA_ECOLI	157	4.14	7.93
PPDA_ECOLI	156	4.13	7.90
SLYB_ECOLI	155	4.12	7.87
YFJT_ECOLI	155	4.12	7.87
YKFB_ECOLI	155	4.12	7.87
NLPC_ECOLI	154	4.11	7.84
YEHR_ECOLI	153	4.11	7.81
YIBG_ECOLI	153	4.11	7.81
YEGJ_ECOLI	153	4.11	7.81
CSGB_ECOLI	151	4.09	7.76
CSGA_ECOLI	151	4.09	7.76
NAPB_ECOLI	149	4.07	7.70
YFJS_ECOLI	147	4.06	7.64
YAFY_ECOLI	147	4.06	7.64
YHHA_ECOLI	146	4.05	7.61
ZRAP_ECOLI	141	4.01	7.46
HSLJ_ECOLI	140	4.00	7.43
CSGF_ECOLI	138	3.98	7.37
YEDD_ECOLI	137	3.98	7.34
HIUH_ECOLI	137	3.98	7.34
YUAE_ECOLI	137	3.98	7.34
YGHG_ECOLI	136	3.97	7.31
YGDB_ECOLI	135	3.96	7.27
RCSF_ECOLI	134	3.95	7.24
YAAI_ECOLI	134	3.95	7.24
YCGK_ECOLI	133	3.94	7.21
YUBK_ECOLI	132	3.93	7.18

YGIW_ECOLI	130	3.92	7.12
YDEI_ECOLI	130	3.92	7.12
FLHE_ECOLI	130	3.92	7.12
CSGE_ECOLI	129	3.91	7.09
RUTC_ECOLI	128	3.90	7.06
YBGS_ECOLI	126	3.88	6.99
YCFL_ECOLI	125	3.87	6.96
YFFQ_ECOLI	125	3.87	6.96
YFEK_ECOLI	124	3.86	6.93
YOBA_ECOLI	124	3.86	6.93
YBAV_ECOLI	123	3.85	6.90
YBBC_ECOLI	122	3.84	6.87
YQJC_ECOLI	122	3.84	6.87
YCGJ_ECOLI	122	3.84	6.87
YFIL_ECOLI	121	3.83	6.83
YEBF_ECOLI	118	3.81	6.74
YJEI_ECOLI	117	3.80	6.70
YACC_ECOLI	115	3.78	6.64
SMPA_ECOLI	113	3.76	6.57
YEBY_ECOLI	113	3.76	6.57
YNFB_ECOLI	113	3.76	6.57
OSME_ECOLI	112	3.75	6.54
YOHN_ECOLI	112	3.75	6.54
HDEA_ECOLI	110	3.73	6.47
CSGC_ECOLI	110	3.73	6.47
CUSF_ECOLI	110	3.73	6.47
YIDQ_ECOLI	110	3.73	6.47
MLIC_ECOLI	109	3.72	6.44
BSMA_ECOLI	109	3.72	6.44
YJDP_ECOLI	109	3.72	6.44
YKGJ_ECOLI	109	3.72	6.44
YMGD_ECOLI	109	3.72	6.44
YBFN_ECOLI	108	3.71	6.40
HDEB_ECOLI	108	3.71	6.40
YPEC_ECOLI	108	3.71	6.40
PTFB1_ECOLI	108	3.71	6.40
YDBL_ECOLI	108	3.71	6.40
YECR_ECOLI	107	3.70	6.37
YFIM_ECOLI	107	3.70	6.37
PSIF_ECOLI	106	3.69	6.33
YEGR_ECOLI	105	3.68	6.30
PSPE_ECOLI	104	3.67	6.27
YMDA_ECOLI	103	3.66	6.23
ASR_ECOLI	102	3.65	6.20
YNFD_ECOLI	101	3.64	6.16
YSAB_ECOLI	99	3.62	6.09
YAAX_ECOLI	98	3.61	6.06
YDAS_ECOLI	98	3.61	6.06
BORD_ECOLI	97	3.60	6.02
YICS_ECOLI	97	3.60	6.02
YDDL_ECOLI	96	3.58	5.99
YUAS_ECOLI	95	3.57	5.95
YBJH_ECOLI	94	3.56	5.91
YEHE_ECOLI	93	3.55	5.88
YPDI_ECOLI	91	3.53	5.81
YJFN_ECOLI	91	3.53	5.81
YJFY_ECOLI	91	3.53	5.81
YAHO_ECOLI	91	3.53	5.81
YNJH_ECOLI	90	3.52	5.77
YDBJ_ECOLI	88	3.49	5.70
YHCN_ECOLI	87	3.48	5.66
YBIJ_ECOLI	86	3.47	5.62
MCBA_ECOLI	86	3.47	5.62
YQHH_ECOLI	85	3.46	5.58

BHSA_ECOLI	85	3.46	5.58
YOAF_ECOLI	84	3.45	5.55
RZOQ_ECOLI	84	3.45	5.55
YJBE_ECOLI	80	3.40	5.39
LPP_ECOLI	78	3.37	5.32
YKGI_ECOLI	78	3.37	5.32
YNCJ_ECOLI	76	3.35	5.24
YGDI_ECOLI	75	3.34	5.20
YCEK_ECOLI	75	3.34	5.20
YHDV_ECOLI	73	3.31	5.12
OSMB_ECOLI	72	3.30	5.08
YGDR_ECOLI	72	3.30	5.08
MARB_ECOLI	72	3.30	5.08
YIFL_ECOLI	67	3.23	4.87
RZOR_ECOLI	61	3.14	4.62
YNBE_ECOLI	61	3.14	4.62
RZOD_ECOLI	60	3.12	4.58
YDCA_ECOLI	57	3.08	4.44
YHFL_ECOLI	55	3.05	4.35
HOKD_ECOLI	51	2.98	4.17
HOKC_ECOLI	50	2.96	4.12
ECNB_ECOLI	48	2.93	4.03
MGRB_ECOLI	47	2.91	3.98
ECNA_ECOLI	41	2.80	3.68

Table S9. **Identity and index of proPhoA peptides used on the peptide arrays**

No	Start aa	Peptide sequence	No	Start aa	Peptide sequence	No	Start aa	Peptide sequence	No	Start aa	Peptide sequence	No	Start aa	Peptide sequence
1	1	MKQSTIALALLPL	36	106	YTHYALNKKTGKP	71	211	GSITEQLLNARAD	106	316	DSVPTLAQMTDKA	141	421	VMSYGNSEEDSQE
2	4	STIALALLPLLFT	37	109	YALNKKTGKPDYV	72	214	TEQLLNARADVTL	107	319	PTLAQMTDKAIEL	142	424	YGNSEEDSQEHTG
3	7	ALALLPLLFTPVT	38	112	NKKTGKPDYVTDS	73	217	LLNARADVTLGGG	108	322	AQMTDKAIELLSK	143	427	SEEDSQEHTGSQL
4	10	LLPLLFTPVTKAR	39	115	TGKPDYVTDSAAS	74	220	ARADVTLGGGAKT	109	325	TDKAIELLSKNEK	144	430	DSQEHTGSQLRIA
5	13	LLFTPVTKARTPE	40	118	PDYVTDSAASATA	75	223	DVTLGGGAKTFAE	110	328	AIELLSKNEKGFF	145	433	EHTGSQLRIAAYG
6	16	TPVTKARTPEMPV	41	121	VTDSAASATAWST	76	226	LGGGAKTFAETAT	111	331	LLSKNEKGFFLQV	146	436	GSQLRIAAYGPHA
7	19	TKARTPEMPVLEN	42	124	SAASATAWSTGVK	77	229	GAKTFAETATAGE	112	334	KNEKGFFLQVEGA	147	439	LRIAAYGPHAANV
8	22	RTPEMPVLENRAA	43	127	SATAWSTGVKTYN	78	232	TFAETATAGEWQG	113	337	KGFFLQVEGASID	148	442	AAYGPHAANVVGL
9	25	EMPVLENRAAQGD	44	130	AWSTGVKTYNGAL	79	235	ETATAGEWQGKTL	114	340	FLQVEGASIDKQD	149	445	GPHAANVVGLTDQ
10	28	VLENRAAQGDITA	45	133	TGVKTYNGALGVD	80	238	TAGEWQGKTLREQ	115	343	VEGASIDKQDHAA	150	448	AANVVGLTDQTDL
11	31	NRAAQGDITAPGG	46	136	KTYNGALGVDIHE	81	241	EWQGKTLREQAQA	116	346	ASIDKQDHAANPC	151	451	VVGLTDQTDLFYT
12	34	AQGDITAPGGARR	47	139	NGALGVDIHEKDH	82	244	GKTLREQAQARGY	117	349	DKQDHAANPCGQI	152	454	LTDQTDLFYTMKA
13	37	DITAPGGARRLTG	48	142	LGVDIHEKDHPTI	83	247	LREQAQARGYQLV	118	352	DHAANPCGQIGET	153	457	QTDLFYTMKAALG
14	40	APGGARRLTGDQT	49	145	DIHEKDHPTILEM	84	250	QAQARGYQLVSDA	119	355	ANPCGQIGETVDL	154	460	DLFYTMKAALGLK
15	43	GARRLTGDQTAAL	50	148	EKDHPTILEMAKA	85	253	ARGYQLVSDAASL	120	358	CGQIGETVDLDEA			
16	46	RLTGDQTAALRDS	51	151	HPTILEMAKAAGL	86	256	YQLVSDAASLNSV	121	361	IGETVDLDEAVQR			
17	49	GDQTAALRDSLSD	52	154	ILEMAKAAGLATG	87	259	VSDAASLNSVTEA	122	364	TVDLDEAVQRALE			
18	52	TAALRDSLSDKPA	53	157	MAKAAGLATGNVS	88	262	AASLNSVTEANQQ	123	367	LDEAVQRALEFAK			
19	55	LRDSLSDKPAKNI	54	160	AAGLATGNVSTAE	89	265	LNSVTEANQQKPL	124	370	AVQRALEFAKKEG			
20	58	SLSDKPAKNIILL	55	163	LATGNVSTAELQD	90	268	VTEANQQKPLLGL	125	373	RALEFAKKEGNTL			
21	61	DKPAKNIILLIGD	56	166	GNVSTAELQDATP	91	271	ANQQKPLLGLFAD	126	376	EFAKKEGNTLVIV			
22	64	AKNIILLIGDGMG	57	169	STAELQDATPAAL	92	274	QKPLLGLFADGNM	127	379	KKEGNTLVIVTAD			
23	67	IILLIGDGMGDSE	58	172	ELQDATPAALVAH	93	277	LLGLFADGNMPVR	128	382	GNTLVIVTADHAH			
24	70	LIGDGMGDSEITA	59	175	DATPAALVAHVTS	94	280	LFADGNMPVRWLG	129	385	LVIVTADHAHASQ			
25	73	DGMGDSEITAARN	60	178	PAALVAHVTSRKC	95	283	DGNMPVRWLGPKA	130	388	VTADHAHASQIVA			
26	76	GDSEITAARNYAE	61	181	LVAHVTSRKCYGP	96	286	MPVRWLGPKATYH	131	391	DHAHASQIVAPDT			
27	79	EITAARNYAEGAG	62	184	HVTSRKCYGPSAT	97	289	RWLGPKATYHGNI	132	394	HASQIVAPDTKAP			
28	82	AARNYAEGAGGFF	63	187	SRKCYGPSATSEK	98	292	GPKATYHGNIDKP	133	397	QIVAPDTKAPGLT			
29	85	NYAEGAGGFFKGI	64	190	CYGPSATSEKCPG	99	295	ATYHGNIDKPAVT	134	400	APDTKAPGLTQAL			
30	88	EGAGGFFKGIDAL	65	193	PSATSEKCPGNAL	100	298	HGNIDKPAVTCTP	135	403	TKAPGLTQALNTK			
31	91	GGFFKGIDALPLT	66	196	TSEKCPGNALEKG	101	301	IDKPAVTCTPNPQ	136	406	PGLTQALNTKDGA			
32	94	FKGIDALPLTGQY	67	199	KCPGNALEKGGKG	102	304	PAVTCTPNPQRND	137	409	TQALNTKDGAVMV			
33	97	IDALPLTGQYTHY	68	202	GNALEKGGKGSIT	103	307	TCTPNPQRNDSVP	138	412	LNTKDGAVMVMSY			
34	100	LPLTGQYTHYALN	69	205	LEKGGKGSITEQL	104	310	PNPQRNDSVPTLA	139	415	KDGAVMVMSYGNS			
35	103	TGQYTHYALNKKT	70	208	GGKGSITEQLLNA	105	313	QRNDSVPTLAQMT	140	418	AVMVMSYGNSEED			