SUPPORTING INFORMATION

Table S1. Plasmids Used in the Study

Plasmid[a]	Construction/Comments[b]	Reference
pT7ecSecA	His-tagged *E. coli secA* gene under T7 promoter control	Zito *et al.*[1]
pT7ecSecA-PhoAss	Insertion of *phoA* signal sequence between codon 901 of *secA* and his-tag of pT7ecSecA in three stages:codons 1-7, 8-15, and 16-21	This study
pT7ecSecA-BamHI-PhoAss	Insertion of *BamHI* site between end of *secA* gene and beginning of *phoA* signal sequence of pT7ecSecA-PhoAss	This study
pCDF-BAD-phoA	*phoA* gene under *araBAD* promoter regulation	Grady *et al.*[2]
pT7ecSecA-PhoA	Fusion of entire *phoA* gene onto end of *secA* gene. For this purpose *XhoI* and *BamHI* sites were added onto the 5' and 3' ends, respectively, of *phoA* by PCR amplification of pCDF-BAD-phoA; the resulting DNA fragment was cleaved appropriately purified and inserted into the corresponding sites of pT7ecSecA-BamHI-PhoAss	This study
pT7ecSecA-PhoA(R14)	Substitution of Arg for Leu14 of pT7ecSecA-PhoA	This study
pT7ecSecA834-PhoA	Deletion of *E. coli secA* codons 835-901 of	This study

	pT7ecSecA-PhoA	
pT7ecSecA834-PhoA(R14)	Substitution of Arg for Leu14 of pT7ecSecA834-PhoA	This study
pT7div	*B. subtilis secA* gene under T7 promoter control	McNicholas *et al.*[3]
pT7div2	Loss of internal *NdeI* site within *B. subtilis secA* gene of pT7div by a synonymous codon substitution	This study
pT7bsSecA-PhoAss	Substitution of *B. subtilis secA* gene for its *E. coli* counterpart in pT7ecSecA-BamHI-PhoAss. For this purpose *NdeI* and *BamHI* sites were added onto the 5' and 3' ends, respectively, of *B. subtilis secA* by PCR amplification of pT7div2. After appropriate cleavage this DNA fragment was purified and used to replace the *E. coli secA* portion of pT7ecSecA-BamHI-PhoAss that had been cleaved similarly	This study
pT7bsSecA	Deletion of *phoA* signal sequence of pT7bsSecA-PhoAss	This study
pT7bsSecA783	Deletion of *B. subtilis secA* codons 784-841 of pT7bsSecA	This study
pT7bsSecA783-PhoAss	Deletion of *B. subtilis secA* codons 784-841 of pT7bsSecA-PhoAss	This study
pT7bsSecA783-GS(2.5)-PhoAss	Insertion of Gly Ser Gly Ser Gly linker between *B. subtilis secA* codon 783 and phoA signal sequence of	This study

	pT7bsSecA783-PhoAss	
pT7bsSecA783-GS(5)-PhoAss	Insertion of additional Gly Ser Gly Ser Gly linker between Gly Ser Gly Ser Gly linker and *phoA* signal sequence of pT7bsSecA783-GS(2.5)-PhoAss	This study
pT7bsSecA783 F652-GS(5)-PhoAss Cys2	Substitution of Phe at *secA* W652 and insertion of Cys as second codon of *phoA* signal sequence of pT7bsSecA783-GS(5)-PhoAss	This study
pT7bsSecA783 F652-GS(5)-PhoAss Cys22	Substitution of Phe at *secA* W652 and insertion of Cys as 22nd codon of *phoA* signal sequence of pT7bsSecA783-GS(5)-PhoAss	This study
pT7bsSecA-KRRLamBss	Insertion of KRR*lamB* signal sequence between codon 841 of *B. subtilis secA* and his-tag of pT7bsSecA in three stages:codons 1-10, 11-20, and 21-28	This study
pT7bsSecA783-KRRLamBss	Deletion of *B. subtilis secA* codons 784-841 of pT7bsSecA-KRRLamBss	This study
pT7bsSecA783-GS(2.5)-KRRLamBss	Insertion of Gly Ser Gly Ser Gly linker between *B. subtilis secA* codon 783 and KRR*lamB* signal sequence of pT7bsSecA783-KRRLamBss	This study
pT7bsSecA783 F652-GS(2.5)-KRRLamBss Cys2	Substitution of Phe at *B. subtilis secA* W652 and insertion of Cys as second codon of KRR*lamB* signal sequence of	This study

	pT7bsSecA783-GS(2.5)-KRRLamBss	
pT7bsSecA783 F652-GS(2.5)-KRRLamBss Cys29	Substitution of Phe at *B. subtilis secA* W652 and insertion of Cys as 29th codon of KRR*lamB* signal sequence of pT7bsSecA783-GS(2.5)-KRRLamBss	This study
pT7bsSecA783-GS(5)-KRRLamBss	Insertion of additional Gly Ser Gly Ser Gly linker between Gly Ser Gly Ser Gly linker and KRR*lamB* signal sequence of pT7bsSecA783-GS(2.5)-KRRLamBss	This study
pT7bsSecA783-LamBss	Deletion of Lys Arg Arg codons 8-10 of KRR*lamB* signal sequence of pT7bsSecA783-KRRLamBss	This study
pT7bsSecA783-GS(2.5)-LamBss	Deletion of Lys Arg Arg codons 8-10 of KRR*lamB* signal sequence of pT7bsSecA783-GS(2.5)-KRRLamBss	This study
pT7bsSecA783-GS(5)-LamBss	Deletion of Lys Arg Arg codons 8-10 of KRR*lamB* signal sequence of pT7bsSecA783-GS(5)-KRRLamBss	This study

[a]All SecA or SecA-signal peptide chimeras constructed in this study contained carboxyl-terminal hexa-histidine tags.

[b]QuikChange™ mutagenesis was utilized for the construction of appropriate insertions, deletions or substitutions in plasmids as described by the manufacturer unless otherwise indicated. Plasmid DNA sequence was verified by the University of Pennsylvania DNA Sequencing Facility.

Figure S1

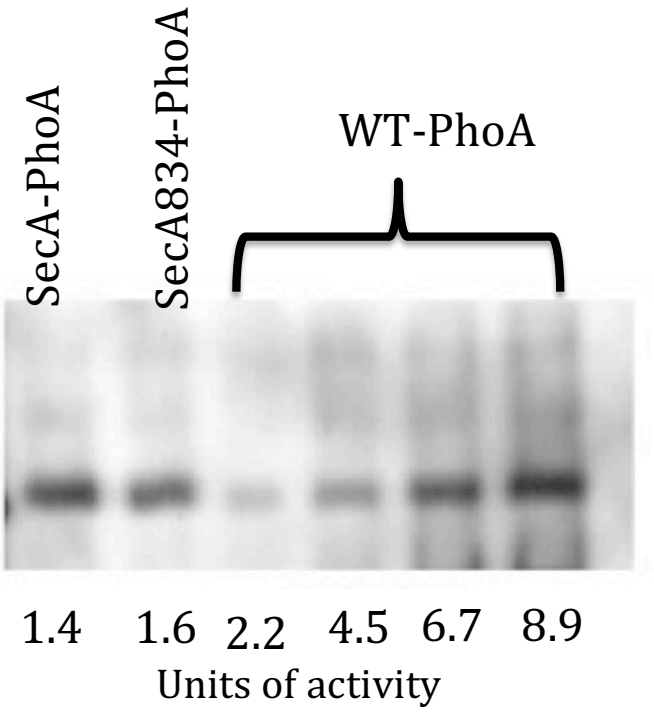

Figure S1. Estimation of Translocation Efficiency of Chimeras Based On Alkaline Phosphatase Specific Activity. BL21.20 containing pT7ecSecA-PhoA or pT7ecSecA834-PhoA or the control plasmids containing defective signal peptides, pT7ecSecA-PhoA(R14) or pT7ecSecA834-PhoA(R14), were grown in LB supplemented with 100 mg/ml of ampicillin at 39°C until an A600 of 0.4, when they were induced with 0.5 mM IPTG for 1 h. As a control, a culture of MC1000 phoR$^-$ that is constitutive for wild-type alkaline phosphatase (WT-PhoA) production was also prepared similarly, but without IPTG induction. Cells were harvested by sedimentation and resuspended in TKM buffer. A portion of each culture was either assayed for alkaline phosphatase activity as described previously [2] or analyzed by western blotting utilizing alkaline phosphatase

antisera. The number of units of alkaline phosphatase activity within each aliquot analyzed on the gel is shown at the bottom of the western blot.

Figure S2.

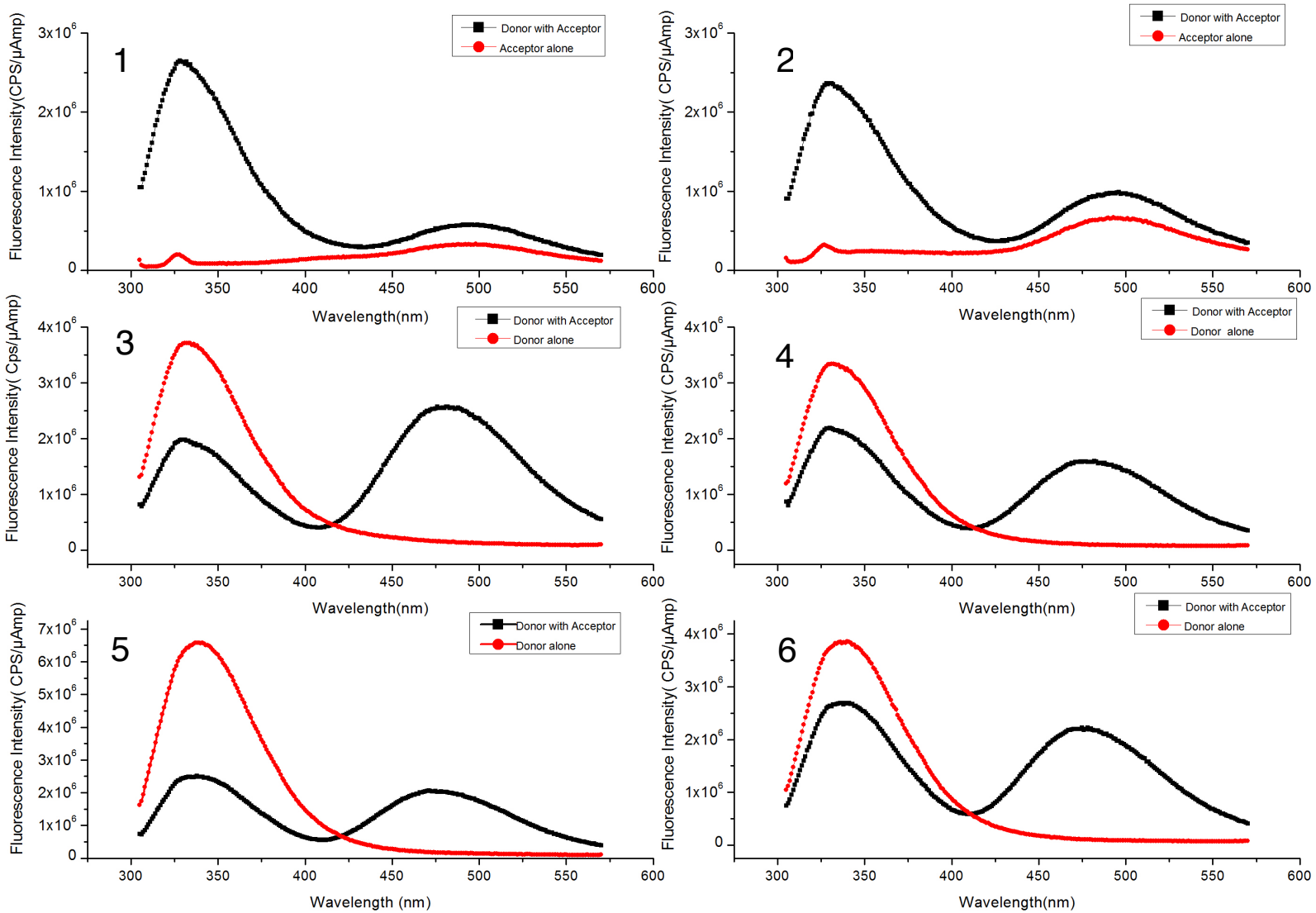
1
Donor with Acceptor
Acceptor alone
Fluorescence Intensity(CPS/μAmp)
Wavelength(nm)
2
Donor with Acceptor
Acceptor alone
Fluorescence Intensity(CPS/μAmp)
Wavelength(nm)
3
Donor with Acceptor
Donor alone
Fluorescence Intensity(Cps/μAmp)
Wavelength(nm)
4
Donor with Acceptor
Donor alone
Fluorescence Intensity(CPS/μAmp)
Wavelength(nm)
5
Donor with Acceptor
Donor alone
Fluorescence Intensity(CPS/μAmp)
Wavelength (nm)
6
Donor with Acceptor
Donor alone
Fluorescence Intensity(CPS/μAmp)
Wavelength(nm)

Figure S2. SecA Trp724 donor quenching and IAEDANS-labeled signal peptide acceptor enhancement for SecA or SecA-Signal Peptide Chimeras. Fluorescence Spectra: (1) IAEDANS-labeled SP2 in the presence or absence of bsSecA783 F652; (2) IAEDANS-labeled SP22 in the presence or absence of bsSecA783 F652; (3) bsSecA783 F652-GS(5)-KRRLamBss Cys2 with or without IAEDANS labeling; (4) bsSecA783 F652-GS(5)-KRRLamBss Cys29 with or without IAEDANS labeling; (5) bsSecA783 F652-GS(10)-PhoAss Cys2 with our without IAEDANS labeling; (6) bsSecA783 F652-GS(10)-PhoAss Cys22 with or without IAEDANS labeling; 1 μM IAEDANS-labeled or unlabeled SecA chimera or 4 μM IAEDANS-labeled SP2 or SP22 in the presence or absence of 1 μM SecA protein was utilized with excitation at 295 nm in all cases. All spectra were background corrected.

Figure S3

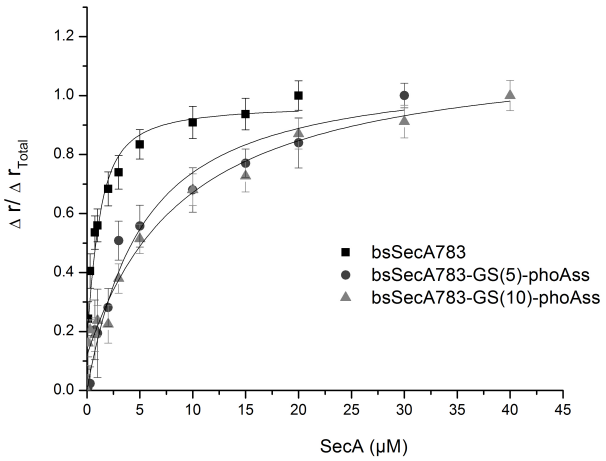

Figure S3: The effect of GS-linker length on the binding affinity of bsSecA783 chimera proteins binding to signal peptide as measured by fluorescence anisotropy. The bsSecA783 exhibits the highest affinity for the exogenous peptide more than 5 times that of the chimera proteins. The K_D values are given in Table 1. Anisotropy measurements were conducted on samples containing 1 μM peptide and titrating in bsSecA783 or the bsSecA783 chimera as described in the experimental procedures. The data are normalized to the total anisotropy change observed for clarity in display.

Figure S4

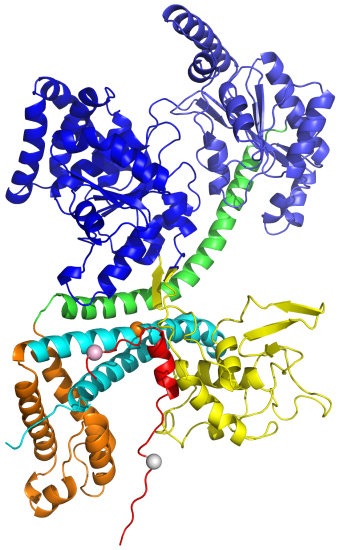

Figure S4: A depiction of *E. coli* SecA with KRRlamB signal peptide bound in a perpendicular fashion based on the NMR structure ((PDB ID: 2VDA)[4]. Color-coding of the SecA domains or sub-domains is as follows: NBD-I (dark blue), NBD-2 (light blue), PPXD (yellow), HSD excluding THF (green), THF (cyan), and HWD (brown). bsSecA Trp724 residue is depicted as an orange space-filling sphere. KRRLamB signal peptide is shown in red with dye labeled residues at the beginning or end of the corresponding signal peptide depicted by grey or pink space-filling spheres, respectively.

REFERENCES

[1] Zito, C., Antony, E., Hunt, J., Oliver, D., and Hingorani, M. (2005) Role of a conserved glutamate residue in the Escherichia coli SecA ATPase mechanism, *J Biol Chem 280*, 14611-14619.

[2] Grady, L., Michtavy, J., and Oliver, D. (2011) Characterization of the Escherichia coli SecA signal peptide-binding site., *J Bacteriol 194*, 307-316.

[3] McNicholas, P., Rajapandi, T., and Oliver, D. (1995) SecA proteins of *Bacillus subtilis* and *Escherichia coli* possess homologous amino-terminal ATP-binding domains regulating integration into the plasma membrane., *J. Bacteriol. 177*, 7231-7237.

[4] Gelis, I., Bonvin, A., Keramisanou, D., Koukaki, M., Gouridis, G., Karamanou, S., Economou, A., and Kalodimos, C. (2007) Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR, *Cell 131*, 756-769.