TABLE S1 Residues selected for *in vivo* photo-crosslinking and summary of results

Domain	Residue[a]	Structural basis or reference	Corresponding residue (chain)[b]	Results of crosslinking
NBD	Leu2	2IPC/(1, 2)	Met1 (C)	SecA dimer and SecA-EF-Tu
	Ile3	Div_dimer/2IPC/(1, 2)	Leu2 (A, B)/Leu2 (C)	SecA dimer and SecA-EF-Tu
	Lys4	Div_dimer/(1, 2)	Gly3 (A, B)	SecA dimer and SecA-EF-Tu
	Leu5	Div_dimer/(1, 2)	Ile4 (A, B)	SecA dimer and SecA-EF-Tu
	Leu6	Div_dimer/(1, 2)	Leu5 (A, B)	SecA dimer and SecA-EF-Tu
	Thr7	Div_dimer/2IBM/2IPC /(1, 2)	Asn6 (A, B)/Asn6 (A)/Arg6 (A, B)	SecA dimer
	Lys8	Div_dimer/2IBM/(1, 2)	Lys7 (A, B)/Lys7 (A)	SecA dimer and SecA-EF-Tu
	Val9	Div_dimer/2IBM/(1, 2)	Met8 (A, B)/Met8 (A)	SecA dimer and SecA-EF-Tu
	Phe10	(1, 2)		SecA dimer
	Gly11	2IPC/(1, 2)	Asp10 (A, B)	SecA dimer and SecA-EF-Tu
	Phe68	–[c]		protein precipitated
	Tyr134	2FSF-PBD	Tyr134 (A, B)	protein precipitated
	Ser402	–[c]		no crosslink
PBD	His262	2FSF-PBD	His262 (B)	no crosslink
	Phe263	2FSF-PBD	Phe263 (A, B)	SecA dimer
	Ser264	2FSF-PBD	Ser264 (A, B)	intramolecular crosslink[d]
	Val265	2FSF-PBD	Val265 (A, B)	no crosslink
	Asp266	2FSF-PBD	Asp266 (A, B)	intramolecular crosslink[d]
	Val272	–[c]		no crosslink
	Thr340	Div_dimer/2IBM	Thr320 (A, B)/Thr320 (B)	no crosslink
IRA2	Thr470	2FSF-PBD/2IBM	Thr470 (A, B)/Lys450 (B)	protein precipitated
	Ile474	–[c]		protein precipitated
	Phe483	2FSF-PBD	Phe483 (A, B)	no crosslink
	Thr530	2FSF-PBD	Thr530 (A, B)	no crosslink
	Phe598	Div_dimer/2IBM	Phe549 (A, B)/Phe549 (A, B)	weak crosslink
SD	Ser636	Div_dimer/2IBM	Gly587 (A, B)/Gly587 (A, B)	no crosslink
	Phe639	Div_dimer/2IBM	Phe590 (A, B)/Phe590 (A)	intramolecular crosslink[d]
	Gln662	Div_dimer	Gln613 (A, B)	no crosslink
	Glu665	Div_dimer	Glu616 (A, B)	no crosslink
IRA1	His790	2IBM	His739 (A, B)	intramolecular crosslink[d]
	Leu791	2IBM/1NL3	Leu740 (B)/Leu795 (A, B)	intramolecular crosslink[d]

(continued)

Domain	Residue[a]	Structural basis or reference	Corresponding residue (chain)[b]	Results of crosslinking
IRA1	Gly793	–[c]		intramolecular crosslink[d]
	Tyr794	2IBM/1NL3	Tyr743 (B)/Met798 (A, B)	SecA dimer and intramolecular crosslink[d]
	Gln796	Div_dimer/2IBM/2IPC	Gln745 (A, B)/Gln745 (A)/ Gln903 (C, D)	intramolecular crosslink[d]
	Asp798	Div_dimer	Asn747 (A, B)	intramolecular crosslink[d]
	Lys800	–[c]		no crosslink
	Gln801	Div_dimer/2IPC	Arg750 (A, B)/Gln908 (C, D)	weak crosslink and intramolecular crosslink[d]
	Tyr803	–[c]		no crosslink
	Lys804	Div_dimer/2IPC	Gln753 (A, B)/Lys911 (C, D)	no crosslink
	Arg805	Div_dimer	Met754 (A, B)	SecA dimer
	Ser807	–[c]		no crosslink
	Phe808	Div_dimer/ (3)	Phe757 (A, B)	weak crosslink
	Ser809	–[c]		weak crosslink and intramolecular crosslink[d]
	Met810	(3)		intramolecular crosslink[d]
	Phe811	Div_dimer/ (3)	Phe760 (A, B)	no crosslink
	Ala812	–[c]		no crosslink
	Met814	(3)		no crosslink
	Leu815	Div_dimer/ (3)	Ile764 (A, B)	weak crosslink and SecA-EF-Tu
	Ser817	–[c]		no crosslink
	Leu818	Div_dimer/ (3)	Ile767 (A, B)	weak crosslink
	Tyr820	–[c]		no crosslink

[a] The indicated *ec*secA residue was amber mutated for *p*Bpa incorporation.

[b] The interface residues in each structure were predicted with the SPPIDER program, and the corresponding *ec*SecA residue was determined based on amino acid sequence alignment (ClustalW, http://www.ebi.ac.uk/Tools/clustalw2/).

[c] The residue was selected without a published account of its possible role in dimerization.

[d] The migration of the band in SDS-PAGE is just slightly slower than monomeric SecA. MS analysis shows no other protein is crosslinked to SecA and Western blot with anti-c-myc antibody suggests the band is SecA monomer.

Figure S1

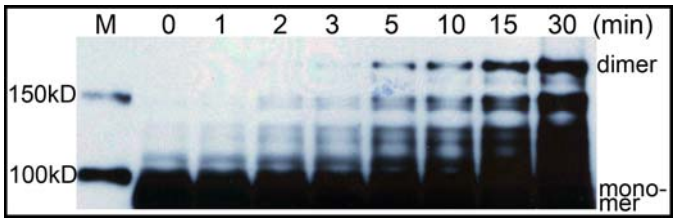

FIG S1 Western blot analysis of SecA L6X subjected to photo-crosslinking for the indicated time. His-antibody was utilized. The positions of SecA monomer and dimer are given. Lane M shows protein molecular mass markers with molecular masses on the left.

Figure S2

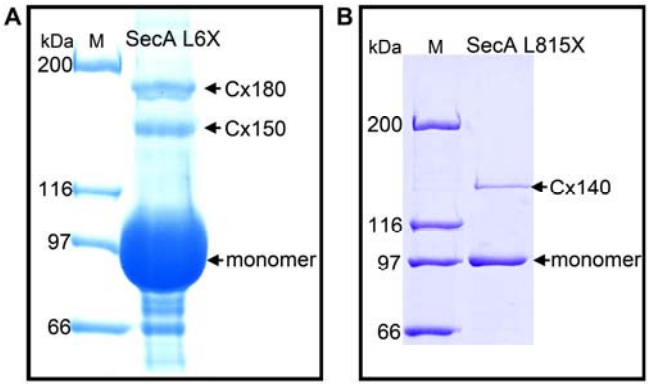

FIG S2 Proteins for LC-MS/MS analysis were purified from UV-treated cells and resolved on 7.5% SDS-PAGE. (A) SecA L6X and its crosslinked products Cx150 and Cx180. The gel was stained with Coomassie G-250. (B) SecA L815X and its crosslinked product Cx140. Three crosslinked bands were separately cut from the gel, digested with trypsin and analyzed with LC-MS/MS. The data indicate that the Cx180 band is SecA dimer and the bands Cx150 and Cx140 represent SecA-EF-Tu complex. Lane M, protein molecular mass markers with molecular masses shown on the left of each panel.

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