Supporting information to

Non-competitive binding of PpiD and YidC to the SecYEG translocon expands the global view on the SecYEG interactome in *E. coli*

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File content:

Table S1 (separate data file): Complete list of putative SecYEG interacting proteins identified by label free proteomics.

Table S2: List of oligonucleotides used for PCR reactions

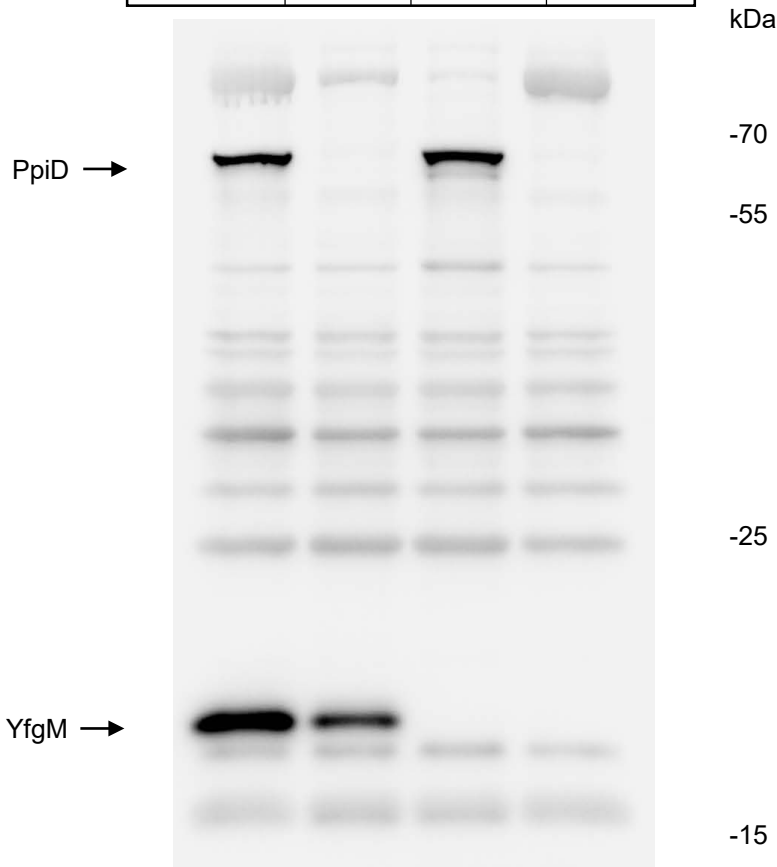**Figure S1:** Significant interactors of the SecYEG in E. coli.

Figure S2: PpiD antibodies recognize both PpiD and YfgM

Table S1 (separate Data file) : Complete List of putative SecYEG interacting proteins identified by label-free proteomics with and without DSS-cross-linking. When indicated, YidC was co-expressed with SecY_{His}EG.

Table S2: List of oligonucleotides used for PCR reactions.

Primer name	5' – oligonucleotides – 3'	Purpose of use
SecY_plug_removal_for	GCTCTCAGCCGTGCTTCTATCTTTGCTC	Removal of SecY plug domain
SecY_plug_removal_rev	GATGGTGCCTCGCTGTTGCTCAAGC	
YidC_cutpBadlong_for	CGCGATCTAGAGGGCTAGCAGGAGGAATTCAC	Cloning of pTrc99a-SecY_{His}EG-YidC
YidC_cutpBadlong_rev	CGGTCGTCGACTCAGGATTTTTTCTTCTCGCG	
YidC_HiR10_for	GGCAGCGGAGATTCGC	Cloning of pTrc99a-SecY_{His}EG-YidC
YidC_HiR10_rev	CATCGTTAGTGTTCTCCATGGTG	
SecY_I61_for	ACCATCTAGGAGATGTTTAAC	Construction of SeY plug (61-70) - pBpA mutants
SecY_I61_rev	GCCTCGCTGTTGCTC	
SecY_E62_for	CGAGGCACCATCATTTAGATGTTTAACATGTTC	
SecY_E62_rev	GAACATGTTAAACATCTAAATGATGGTGCCTCG	
SecY_M63 _for	GAGGCACCATCATTGAGTAGTTTAACATGTTCTCTGG	
SecY_M63_rev	CCAGAGAACATGTTAAACTACTCAATGATGGTGCCTC	
SecY_F64_for	CACCATCATTGAGATGTAGAACATGTTCTCTGGTG	
SecY_F64_rev	CACCAGAGAACATGTTCTACATCTCAATGATGGTG	
SecY_N65_for	CATCATTGAGATGTTTAGATGTTCTCTGGTGGTG	
SecY_N65_rev	CACCACCAGAGAACATCTAAAACATCTCAATGATG	
SecY_M66_for	CATTGAGATGTTTAACTAGTTCTCTGGTGGTGCTC	
SecY_M66_rev	GAGCACCACCAGAGAACTAGTTAAACATCTCAATG	
SecY_F67_for	AACATGTAGTCTGGTGGTGCT	
SecY_F67_rev	AAACATCTCAATGATGGTGCC	
SecY_S68_for	GATGTTTAACATGTTCTAGGGTGGTGCTCTCAGCC	
SecY_S68_rev	GGCTGAGAGCACCACCCTAGAACATGTTAAACATC	
SecY_G69_for	GTTTAACATGTTCTCTTAGGGTGCTCTCAGCCGTG	
SecY_G69_rev	CACGGCTGAGAGCACCCTAAGAGAACATGTTAAAC	
SecY_G70_for	CATGTTCTCTGGTTAGGCTCTCAGCCGTG	
SecY_G70_rev	CACGGCTGAGAGCCTAACCAGAGAACATG	

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Supporting Figure 1: Significant interactors of SecYEG in *E. coli*. (**A**)
SecYEG or SecY$_{His}$EG were expressed in *E. coli*, and subsequently,
SecYEG/SecY$_{His}$EG were purified via metal affinity chromatography and
subjected to digestion using trypsin followed by LC-MS analyses. Volcano
plot representing negative logarithmic p-values from the t-test across replicate
experiments (n=4) plotted against the mean log$_{10}$ intensity ratio of the
respective protein in the SecY$_{His}$EG (His) / SecYEG (Ctl) pull-downs. A
hyperbolic curve (black dashed line) separates specific interactors of the
SecYEG complex marked by green dots from unspecifically copurified
proteins (grey dots). The curves are defined by a bend of 0.25, p-value
threshold of 0.05 and log$_{10}$ ratio limit of 10. Parameters were chosen such that
a false positive rate <0.01 was obtained with the left branch (ratios <1) in (A)
and (B). (**B**) As in (A) but protein-protein-interactions were stabilized by on-
column cross-linking using the chemical cross-linker DSS. A complete list of
all proteins is given in Supporting Table S1.

Supporting Figure 2: PpiD antibodies recognize both PpiD and YfgM. Cells of the indicated *E. coli* strains were TCA precipitated and after SDS-PAGE/Western-blotting decorated with α-PpiD antibodies. Indicated are PpiD and YfgM and the corresponding gel areas are identical to those displayed in Fig. 5F