

Supplementary file 2. HDX data summary table for $\Delta\text{HDX} = (\text{SecYEG} + \text{SecA}^{\text{AMPPNP}}) - (\text{SecYEG} + \text{SecA}^{\text{ADP}})$. Data is presented in **Figure 3**. Mass spectrometry raw data are deposited in the ProteomeXchange Consortium via the PRIDE database.

$\Delta\text{HDX} = (\text{SecYEG} + \text{SecA}^{\text{AMPPNP}}) - (\text{SecYEG} + \text{SecA}^{\text{ADP}})^*$		
	SecY (+ SecA^{AMPPNP})	SecY (+ SecA^{ADP})
HDX reaction details	20 mM Tris pH 8, 2 mM MgCl ₂ , 50 mM KCl, and 0.02% DDM	
HDX time course (min)	0.25, 1, 5 and 30 minutes	
Back-exchange (mean/IQR)	ND	
Number of peptides	137	137
Sequence coverage	91.6	91.6
Average peptide length / Redundancy	2.91	2.91
Replicates (biological or technical)	3 (technical)	3 (technical)
Repeatability (average SD)	0.099	0.093
Significant differences in 30 min ΔHDX^{**}	CI 99% = 0 ± 62 Da	
	SecE (+ SecA^{AMPPNP})	SecE (+ SecA^{ADP})
HDX reaction details	20 mM Tris pH 8, 2 mM MgCl ₂ , 50 mM KCl, and 0.02% DDM	
HDX time course (min)	0.25, 1, 5 and 30 minutes	
Back-exchange (mean/IQR)	ND	
Number of peptides	31	31
Sequence coverage	79.4	79.4
Average peptide length / Redundancy	2.34	2.34
Replicates (biological or technical)	3 (technical)	3 (technical)
Repeatability (average SD)	0.099	0.079
Significant differences in 30 min ΔHDX^{**}	CI 99% = 0 ± 63 Da	
	SecG (+ SecA^{AMPPNP})	SecG (+ SecA^{ADP})
HDX reaction details	20 mM Tris pH 8, 2 mM MgCl ₂ , 50 mM KCl, and 0.02% DDM	
HDX time course (min)	0.25, 1, 5 and 30 minutes	
Back-exchange (mean/IQR)	ND	
Number of peptides	25	25
Sequence coverage	95.5	95.5
Average peptide length / Redundancy	2.11	2.11
Replicates (biological or technical)	3 (technical)	3 (technical)
Repeatability (average SD)	0.101	0.112
Significant differences in 30 min ΔHDX^{**}	CI 99% = 0 ± 64 Da	

* SecA is in excess (mixture of bound and unbound, therefore data not used).

** To compare significant differences, a T-test with $\alpha=0.01$ was used. Only peptides which satisfied a ΔHDX confidence interval of 99 % were considered significant