

Supplementary file 1A. HDX data summary table for $\Delta\text{HDX} = (\text{SecA}^{\text{AMPPNP}}) - (\text{SecA}^{\text{ADP}})$. Data is presented in **Figure 2b**. Mass spectrometry raw data are deposited in the ProteomeXchange Consortium via the PRIDE database.

$\Delta\text{HDX} = (\text{SecA}^{\text{AMPPNP}}) - (\text{SecA}^{\text{ADP}})$		
	SecA^{AMPPNP}	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	215	215
Sequence coverage	81.8	81.8
Average peptide length / Redundancy	2.7	2.7
Replicates (biological or technical)	3 (technical)	3 (technical)
Repeatability (average SD)	0.072	0.07
Significant differences in 30 min ΔHDX^{**}	CI 99% = 0 ± 44 Da	

** 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.

Supplementary file 1B. HDX data summary table for $\Delta\text{HDX} = (\text{SecA}^{\text{AMPPNP}} + \text{SecYEG}) - (\text{SecA}^{\text{ADP}} + \text{SecYEG})$. Data is presented in **Figure 2c**. Mass spectrometry raw data are deposited in the ProteomeXchange Consortium via the PRIDE database.

$\Delta\text{HDX} = (\text{SecA}^{\text{AMPPNP}} + \text{SecYEG}) - (\text{SecA}^{\text{ADP}} + \text{SecYEG})$ *		
	SecA^{AMPPNP} (+ SecYEG)	SecA^{ADP} (+SecYEG)
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	215	215
Sequence coverage	81.8	81.8
Average peptide length / Redundancy	2.7	2.7
Replicates (biological or technical)	3 (technical)	3 (technical)
Repeatability (average SD)	0.064	0.072
Significant differences in 30 min ΔHDX^{**}	CI 99% = 0 ± 41 Da	

* SecYEG were 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.