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online data

SUPPLEMENTARY ONLINE DATA

The dynamic action of SecA during the initiation of protein translocation

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MATERIALS AND METHODS

Steady-state ATPase and determination of the K_m

Steady-state SecA ATPase measurements were performed as described previously [1,2] with various concentrations of ATP. The K_m and V_{max} (k_{cat}) were determined by fitting the data to the Michaelis–Menten equation (eqn S1)

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

where v *is* equal to the enzyme velocity, V_{max} is the total capacity of the substrate-associated ATPase stimulation, [S] is the substrate concentration and K_m is the Michaelis constant.

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Figure S1 Michaelis–Menten parameters for ATP binding

(**A**) ATPase rates of SecA (0.75 μM) over a range of ATP concentrations with: the clamp held in the closed state (black dotted trace), the clamp released from the closed (c) state (silver continuous trace, + 10 mM DTT) and the same released form in the presence of saturating SecYEG (detergent solubilized; 16 μM) (grey dashed trace). (**B**) ATPase assays were performed as in (**A**) using SecA with: the clamp open (black dotted trace) and released from the either the closed (c) or open (o) states (silver continuous trace and grey dashed trace respectively). (**C**) Data in (**A**) and (**B**) were fitted to the Michaelis–Menten equation and the parameters are shown in the histogram (also in Table S1, columns 5 and 6).

Table S1 Kinetic parameters

Various parameters derived from data fits (see individual Figure legends in the main text). The data for the clamp-closed SecA are shown in bold to emphasize the significant differences in this version. Errors represent S.D. values from the fit.

	SecA–SecYEG$_{soluble}$		SecA–SecYEG$_{CL}$		SecA–ATP		SecA$_{(2HF)}$–SecY$_{K268FI}$EG$_{soluble}$		SecA–SecYEG$_{vesicles}$		[SecA-pOA]–SecYEG$_{vesicles}$		[SecA-SecYEG$_{vesicles}$]–pOA	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Condition	K_d (μM)	k_{cat}	K_d (nM)	k_{cat}	K_m (μM)	k_{cat}	K_d (nM)	ΔFI (%)	K_d (μM)	k_{cat}	K_d (μM)	k_{cat}	K_d (μM)	k_{cat}
Δcys	7.6 $\pm$ 0.6	31.4 $\pm$ 0.6	3.7 $\pm$ 1.9	74.9 $\pm$ 3.5	–	–	18.4 $\pm$ 0.6	35.3 $\pm$ 0.2	1.13 $\pm$ 0.32	154.2 $\pm$ 22.0	0.26 $\pm$ 0.04	656.4 $\pm$ 31.0	0.50 $\pm$ 0.12	605.8 $\pm$ 38.3
Δcys + DTT	7.8 $\pm$ 1.4	25.0 $\pm$ 1.1	5.6 $\pm$ 0.6	82.7 $\pm$ 0.9	–	–	20.6 $\pm$ 2.1	36.0 $\pm$ 1.3	1.11 $\pm$ 0.26	149.4 $\pm$ 17.2	0.19 $\pm$ 0.03	631.6 $\pm$ 22.6	0.78 $\pm$ 0.10	604.6 $\pm$ 20.2
Clamp closed	**0.015 $\pm$ 0.011**	**23.2 $\pm$ 0.6**	–	–	**1.7 $\pm$ 0.06**	**8.8 $\pm$ 0.08**	**28.6 $\pm$ 5.0**	**20.9 $\pm$ 1.4**	**0.029 $\pm$ 0.007**	**99.7 $\pm$ 3.0**	**0.025 $\pm$ 0.007**	**131.7 $\pm$ 4.5**	–	–
Clamp released (c)	6.3 $\pm$ 0.7	22.5 $\pm$ 0.7	8.8 $\pm$ 2.1	59.1 $\pm$ 1.9	0.11 $\pm$ 0.02	0.54 $\pm$ 0.02	117.7 $\pm$ 12.7	23.5 $\pm$ 0.8	0.34 $\pm$ 0.20	59.4 $\pm$ 8.2	0.13 $\pm$ 0.02	253.6 $\pm$ 10.0	0.24 $\pm$ 0.06	231.6 $\pm$ 13.3
Clamp open	5.9 $\pm$ 2.1	9.9 $\pm$ 0.5	–	–	0.10 $\pm$ 0.03	0.43 $\pm$ 0.02	60.9 $\pm$ 10.0	15.0 $\pm$ 0.6	0.65 $\pm$ 0.78	22.5 $\pm$ 7.8	0.17 $\pm$ 0.18	21.1 $\pm$ 3.5	–	–
Clamp released (o)	6.0 $\pm$ 1.4	15.9 $\pm$ 0.6	6.5 $\pm$ 0.9	31.2 $\pm$ 0.4	0.23 $\pm$ 0.08	0.63 $\pm$ 0.05	59.5 $\pm$ 6.4	21.3 $\pm$ 0.6	0.65 $\pm$ 0.42	29.9 $\pm$ 6.4	0.13 $\pm$ 0.07	90.3 $\pm$ 9.7	0.35 $\pm$ 0.15	50.5 $\pm$ 4.7
Clamp released (c) + SecYEG	–	–	–	–	1.8 $\pm$ 0.13	8.5 $\pm$ 0.17	–	–	–	–	–	–	–	–

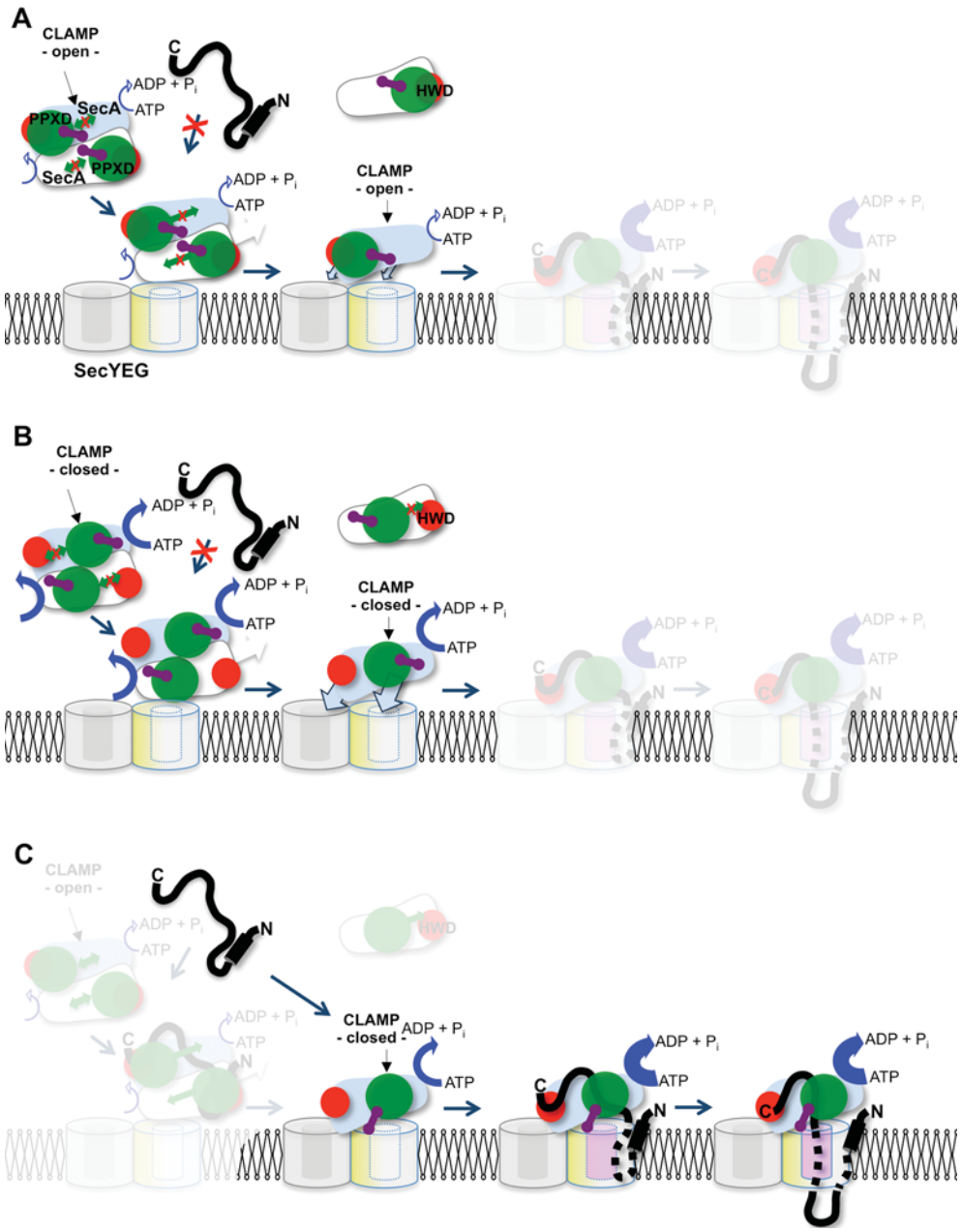

Figure S2 The consequences of the immobilization of the PPXD within SecA to protein transport

The model of pre-protein transport (Figure 7A of the main text) has been modified to incorporate the intra- and inter-molecular disulfide bonds, and to explain their effect on the activity. Colour co-ordination and labelling is as in Figure 7 of the main text. Stages of the mechanism precluded as a result have been fogged out. (**A**) When the PPXD is cross-linked (purple bar) to the HSD the clamp is permanently held open. This prevents the activation of the ATPase activity (thin blue arrows), maintains a low affinity for SecYEG (small pale blue arrows) and prevents association with the pre-protein. (**B**) Fixing the PPXD to NBD2, holding the clamp closed, activates the ATPase activity (blue arrows), brings about a high-affinity association with SecYEG (large pale blue arrows), but prevents the engagement of pre-protein. (**C**) The pre-activated cross-linked SecA–SecYEG complex is primed for translocation and capable of pre-protein intercalation and ATP-driven (thick blue arrows) translocation.

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