HDX-MS reveals nucleotide-dependent, anti-correlated opening and closure of SecA and SecY channels of the bacterial translocon

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Keywords: Hydrogen-deuterium exchange mass spectrometry, conformational dynamics, SecA, SecYEG, protein translocation
Abstract

The bacterial Sec translocon is a multi-protein complex responsible for translocating diverse proteins across the plasma membrane. For post-translational protein translocation, the Sec-channel – SecYEG – associates with the motor protein SecA to mediate the ATP-dependent transport of pre-proteins across the membrane. Previously, a diffusional based Brownian ratchet mechanism for protein secretion has been proposed [Allen et al. eLife 2016;5:e15598]; the structural dynamics required to facilitate this mechanism remain unknown. We employ hydrogen-deuterium exchange mass spectrometry (HDX-MS) to reveal striking nucleotide-dependent conformational changes in the Sec protein-channel from Escherichia coli. In addition to the ATP-dependent opening of SecY, reported previously, we observe a counteracting, and ATP-dependent, constriction of SecA around the pre-protein. ATP binding causes SecY to open and SecA to close; while, ADP produced by hydrolysis, has the opposite effect. This alternating behaviour could help impose the directionality of the Brownian ratchet for protein transport through the Sec machinery.
Introduction

Protein transport across and into the membranes that surround and sub-divide cells is essential for life. The ubiquitous Sec translocon performs this task in the plasma membrane of bacteria and the endoplasmic reticulum of eukaryotes. A protein-channel through the membrane is formed by the conserved hetero-trimeric core-complex: Sec61αβγ in eukaryotes, and SecYEG in archaea and bacteria. In bacteria, protein secretion is important for cell envelope biogenesis and maintenance, as well as for the delivery of adherence and pathogenic effector proteins to the cell surface. During protein secretion, SecYEG engages with the cytosolic motor ATPase SecA, and together they pass pre-proteins with a short N-terminal cleavable signal sequence across the membrane, whilst still in an unfolded conformation.

A structure of the complete SecA-SecYEG complex has been determined with an ATP analogue (ADP-BeF₃; PDB code 3DIN), as well as with a short pre-protein mimic (PDB code 5EUL; modified in Figure 1a). The key regions of SecA and SecYEG are highlighted in the context of this model, shown by orthogonal views for clarity (Figure 1a, b). No structure of the ADP-bound state has been experimentally determined, however molecular dynamics (MD) simulations and Förster Resonance Energy Transfer (FRET) analyses suggest that ATP and ADP respectively favour open and closed forms of the SecY channel.

This behaviour was incorporated into a ‘Brownian ratchet’ model to describe the mechanism of ATP driven protein translocation, whereby ATP binding and hydrolysis act to bias pre-protein diffusion in an outward direction. This directionality is further augmented by other factors, including the promotion of pre-protein folding on the outside, but not the inside, and by coupling transport to the trans-membrane proton-motive-force (PMF).
One criticism of the Brownian ratchet model is the effect of non-hydrolysable ATP analogues: while they cause the channel through SecY to open-up\textsuperscript{8}, they have also been shown to prevent backsliding of trapped translocation intermediates\textsuperscript{11,12}. If the pre-protein channel opens in response to ATP, one might reasonably expect backsliding to occur more readily – not less. To explain this apparent contradiction, one could invoke a second clamp that tightens when ATP is bound and prevents backwards diffusion. Indeed, SecA is known to form a clamp for the translocating pre-protein\textsuperscript{13}, so it could plausibly fulfil this role. Thus far, however, experimental evidence for this idea is lacking. Furthermore, as the SecA clamp can be fixed in a closed state without preventing transport\textsuperscript{14} the conformational changes involved are likely to be subtle, and not necessarily captured with techniques such as FRET.

Clearly, the key to understanding the mechanism that prevents unfavourable back-sliding and refinement of the Brownian ratchet model is to learn more about the dynamic action of SecA-SecYEG at different stages of the ATP hydrolytic cycle. In this study, we exploit recent technical advances in HDX-MS to probe the structural differences between the ATP and ADP associated states that underpin function.

HDX-MS has emerged as a non-invasive and highly sensitive method for interrogating the conformational dynamics of membrane proteins and their complexes\textsuperscript{15-18}. HDX-MS measures the exchange of backbone amide hydrogen to deuterium at peptide-level resolution\textsuperscript{19-21}. The rate of HDX depends on the solvent accessibility, protein flexibility, and hydrogen bonding networks.\textsuperscript{22} Thus, HDX-MS can monitor the conformational changes of proteins complexes by comparing HDX rates between distinct protein states (\textit{e.g.} protein \textit{versus} protein with ligand). Consequently, comparative HDX-MS has emerged as an attractive tool to define conformational changes to complement other, high-resolution techniques. For example, the
analysis of the structural consequences of protein-ligand/ drug interactions, nucleotide dependent protein dynamics; ATP/ GTP versus ADP/ GDP\textsuperscript{23,24}; including those associated with membrane proteins and complexes\textsuperscript{15,17,18}. Here, we use HDX-MS to systematically interrogate nucleotide-induced conformational changes in the multi-component SecA-SecYEG complex.

Results

To investigate the conformational dynamics of SecA, we prepared samples of the enzyme either alone or saturated with an excess of purified SecYEG. We used HDX-MS to interrogate the conformational dynamics of the complex. In each case, the experiments were run in the presence of ADP or a non-hydrolysable analogue of ATP (AMPPNP), and only the data for the saturated component (SecA) was used. AMPPNP was used to interrogate the ATP-bound state because ATP is rapidly hydrolysed to ADP in the active complex: all mentions hereafter of ATP pertinent to the HDX-MS experiments refer to AMPPNP.

Optimised procedures for HDX-MS were carried out as described previously\textsuperscript{9,17} (Figure 1c). The peptide coverage of the whole complex was high (ranging from 82 % to 95% for the respective subunits; Figure 2-figure supplement 1), enabling a thorough analysis of SecA and SecYEG. The comparison of the AMPPNP versus ADP datasets produced a differential HDX-MS fingerprint ($\Delta$HDX) that allowed direct comparisons between the results obtained for distinct states (AMPPNP - ADP; Figure 1c). To interpret the data, we utilised the Wood’s plot, which displays the length and the beginning and end residues of each analysed peptide against their respective $\Delta$HDX. Wood’s plots were prepared using our in-house software (Deuteros\textsuperscript{25}).
We began by investigating the influence of nucleotides on the conformational dynamics of SecA. ΔHDX experiments were performed allowing us to track structural changes between AMPPNP and ADP bound states (SecA\textsuperscript{AMPPNP} – SecA\textsuperscript{ADP}). Surprisingly, there was very little effect on the conformational dynamics of the isolated SecA (Figure 2a, b and Figure 2-figure supplement 2a). The greatest difference in deuterium uptake between the two states was observed at and around the ATP-binding sites (Figure 2a, b). This highlights the fact that SecA is only activated when it is associated with SecYEG; studying SecA by itself is therefore unlikely to yield information pertinent to transport.

To explore the mechanism of protein transport we carried out equivalent comparative HDX-MS experiments in the presence of an excess of SecYEG to saturate SecA (SecA\textsuperscript{AMPPNP}SecYEG - SecA\textsuperscript{ADP}SecYEG) (Figure 2a, c and Figure 2-figure supplement 2b). The results are consistent with SecYEG having a profound effect on SecA: SecYEG induces a large ATP-dependent stabilisation across almost the entirety of SecA (Figure 2c; negative ΔHDX, blue bars). We also examined the conformational effects of SecYEG binding to SecA (Figure 2-figure supplement 3). Interestingly, the results suggested small differences as compared to the differences observed between ATP and ADP bound states of SecA whilst bound to SecYEG (Figure 2c and Figure 2-figure supplement 3). This implies that the presence of nucleotides conformationally activate SecA.

For mechanistic interpretation, the data were analysed in the context of a model of the SecA-SecYEG bound to a short pre-protein\textsuperscript{26} (Figure 2a). Most striking was the correspondence of the stabilised regions with sites in SecA that directly contact the mature regions (PP-M) of the translocating pre-protein (Figure 2a, c; dashed boxes –PP-M, gray bars). Such stabilisation in SecA suggests an ATP-driven conformational closure around the mature...
segment of the pre-protein. Conversely, of course, the highlighted regions identified in SecA would be destabilised in the ADP-bound state, following ATP hydrolysis.

To explore the nucleotide dependent conformational dynamics of the complex we compared the behaviour of SecA to the associated channel complex SecYEG. Previously, we conducted ΔHDX analysis of SecYEGA, as above, but instead saturated SecYEG with SecA⁹. In that instance we were only interested in select peptides lining the cytosolic half of the protein-channel. Here, by taking into account global ΔHDX (SecA⁵⁰⁰⁰PM⁰⁰⁰SecYEG - SecA⁵⁰⁰⁰⁰₇₈SecYEG), we highlight the long-range nucleotide dependent impact of SecA on the dynamics of SecYEG (30 min deuterium exchange; not previously shown⁹; Figure 3).

Interestingly, we found significant ATP-dependent destabilisation of SecY (Figure 3a, b, and Figure 3-figure supplement 1). Closer inspection of the identified peptides reveals that two prominently destabilised regions in the SecY protein would be in direct contact with the signal sequence (PP-S) and mature regions (PP-M) of the pre-protein during protein translocation (Figure 3b; dashed boxes –PP-M gray, and PP-S yellow bars). These regions include the plug, which in the absence of the pre-protein maintains the closed state of the channel²⁷, and the highly conserved trans-membrane helix 7. Multiple regions within the SecE and SecG subunits were also significantly destabilised in the presence of AMPPNP (Figure 3a, c-d and Figure 3-Supplement 1). Again, conversely those identified regions would be stabilised after hydrolysis to ADP. These observations are consistent with an ATP-driven opening of the SecY channel, and closure after hydrolysis⁷,⁸.

The ΔHDX analysis describes contrasting behaviour between the protein channel and cytoplasmic motor components of the bacterial translocon, during the ATP binding and
hydrolysis cycle. Notably, there is an ATP-dependent closure around the translocating polypeptide by SecA and opening of the channel through SecYEG (Figure 4a); reversed in the ADP bound state after ATP hydrolysis (Figure 4b). To further understand this effect with respect to local structural rearrangements in SecA, we re-analysed previously-run all-atom molecular dynamics (MD) simulations of the SecA-SecYE complex engaged with a stretch of pre-protein. The size of two distinct pores in the SecA pre-protein channel through which the pre-protein passes were quantified (Figure 4c, d; see also Methods for details); note that both pores contain residues shown by HDX-MS to be protected in the ATP-bound state (Figure 2, Figure 4c and Methods).

From the simulation data, it is clear that both SecA pores, particularly pore 1 at the cytoplasmic entrance of SecA, are more constricted in the ATP-bound state (Figure 4c, d). This is consistent with the HDX data and suggests that ATP-binding is causing SecA to close around the engaged pre-protein.

Discussion

Overall, our results reveal profound nucleotide dependent differences in the structure of the active Sec complex (SecA-SecYEG). These differences can broadly be divided into two groups: those affecting the membrane channel (SecY, SecE and SecG), which become destabilised in the presence of ATP, and those affecting SecA, which become stabilised. A more detailed analysis reveals that the most dramatic changes affect regions that would directly contact a translocating pre-protein. These results are consistent with the previously proposed Brownian ratchet mechanism of pre-protein transport. The cyclical ATP/ADP dependent conformational dynamics highlighted in SecA and SecYEG are presumably integral components of the underlying mechanism of this ratchetting machine. For instance,
the opening (ATP) and closure (ADP) of the channel through SecY⁹. Secondly, the conferral of ATP dependent asymmetry that prevents folding at the cytosolic side of the membrane, while allowing it at the exterior⁹ are likely to also be a result of these changes. Finally, the observation that ATP causes SecA to constrict around the pre-protein, could potentially explain why pre-proteins do not backslide in the presence of a non-hydrolysable ATP analogue¹¹,¹², and thereby promote forward transport.

Overall, the experiments presented here demonstrate the power of combining HDX-MS – with the results of biochemistry and molecular simulations to understand the conformational mechanism of multi-component membrane protein complexes, difficult to study by other approaches.
Figure legends

Figure 1: Structure of SecA-SecYEG complex and HDX-MS workflow

(a) Structure and sub-domains of SecA-SecYEG (based in 3DIN\textsuperscript{13} modelled with a pre-protein\textsuperscript{26}). The pre-protein, ATP and signal sequence are highlighted in grey, orange and yellow, respectively. (b) Top view of the complex highlighting the nucleotide-binding-domains NBD1 and NBD2 as well as PPXD (Pre-Protein cross-linking Domain) and HWD (Helical Wing Domain) (c) Overview of the HDX process. The sample is prepared in detergent micelles (DDM) and after addition of nucleotides is incubated in a deuterated solvent (1). Following deuteration of the mixture at different time-points, the HDX reaction is quenched and the protein is digested with pepsin (2). Peptides are separated by liquid chromatography and subsequently identified by mass spectrometry (MS). The mass uptake of the protein in different conditions (\textit{e.g.} AMPPNP vs ADP) is then compared (3). Peptides with significant difference in deuterium uptake are mapped onto a Wood’s plot (4). The length of lines represent the peptide sizes. Blue and red regions indicate significant protection and deprotection, respectively. Insignificant differences, calculated as 99% confidence interval, are shown in grey.

Figure 2: Impact of nucleotides on the conformational dynamics of SecA.

(a) Structures of the complex highlighting the contact sites (cyan) in SecA with SecG (green), SecY (pink), mature regions of the pre-protein (PP-M; grey) and ATP (orange). Significant sum differences in relative deuterium uptake ($\Delta$HDX = AMPPNP - ADP) of (b) SecA without SecYEG, and (c) of SecA in the presence of a molar excess of SecYEG after
30 minutes of deuteration. Highlighted regions represent contacts with SecG, SecY, pre-protein (mature domain, PP-M) and ATP; coloured according to (a). Detailed information of HDX-MS data is provided in Supplementary files 1a and 1b.

**Figure 2-figure supplement 1: Linear protein coverage maps for SecA, SecY, SecE and SecG.**

Maps were generated using Deuteros\textsuperscript{25}.

**Figure 2-figure supplement 2: Wood’s plots illustrating the impact of nucleotides on the conformational dynamics of SecA.**

Wood’s plots comparing the relative deuterium uptake (AMPPNP-ADP) of (a) SecA alone and (b) SecA in complex (and saturated) with SecYEG after incubation at 25°C in deuterated solvent for 15 seconds, 1, or 5 minutes. The dotted lines represent the 99% confidence interval, which indicates the level of difference of deuterium uptake between the two compared states is statistically significant. Bars represent individual peptides. Bar length corresponds to peptide size. Red and blue coloured bars indicate statistically significant deprotected or protected peptides, respectively.

**Figure 2-figure supplement 3: Impact of SecYEG binding on SecA.**

Wood’s plots comparing the relative deuterium uptake of SecA upon interaction with SecYEG (present in excess) at 15 seconds, 1, 5 or 30 minutes. Results indicate an overall deprotection, hence destabilisation of SecA upon SecYEG binding.

**Figure 3: Impact of nucleotides on the conformational dynamics of SecY, SecE and SecG.** (a) Structures of the SecA-SecYEG complex highlighting the contact sites of SecY (pink) and SecG (green) with SecA (cyan), signal sequence (PP-S; yellow) and pre-protein
Significant sum differences in relative deuterium uptake ($\Delta$HDX) of (b) SecY, (c) SecE and (d) SecG in the presence of excess SecA at 30 minutes of deuteration. Regions interacting with SecY (b) and SecG (d) are highlighted: SecA, pre-protein mature domain (PP-M) and pre-protein signal sequence (PP-S); colour coding as in (a). Confidence intervals (CI; 99%) are shown as grey dotted lines. Red and blue bars indicate structural stabilisation (positive $\Delta$HDX) and destabilisation (negative $\Delta$HDX) of peptides, respectively. Grey bars indicate peptides with insignificant $\Delta$HDX. Detailed information of HDX-MS data is provided in Supplementary file 2.

Figure 3-figure supplement 1. Wood’s plots illustrating the impact of nucleotides on the conformational dynamics of SecY, SecE and SecG.

Wood’s plots comparing the relative deuterium uptake of SecYEG while bound to SecA (AMPPNP-ADP), showing: (a) SecY (b) SecE and (c) SecG after incubation at 25°C in deuterated solvent for 15 seconds, 1, or 5 minutes. The dotted lines represent the 99% confidence interval, which indicates the level of difference of deuterium uptake between the two compared states is statistically significant. Bars represent individual peptides. Bar length corresponds to peptide size. Red and blue coloured bars indicate statistically significant deprotected or protected peptides, respectively.

Figure 4: ATP-regulation of channel size

(a) Periplasmic, membrane perpendicular and cytosolic views of the SecA-SecYEG-preprotein complex model, with $\Delta$HDX of the peptides mapped onto the structure. The translocon complex is coloured light grey, with H-bonding destabilised and stabilised regions in red and blue, respectively. The pre-protein is shown in yellow (signal sequence) and dark grey (mature). ATP is shown as orange spheres. (b) Schematic summarising the primary
outcomes from the data here. (c) Snapshots of SecA-SecYE with an engaged pre-protein after 1 μs all-atom MD simulation. SecA-SecYE is shown as grey surface and has been slabbed to show the pre-protein channel through both SecA and SecY. The pre-protein is shown as red cartoon, and was absent for the cavity size analysis in (d). The positions of the conserved SecA pores have been highlighted; pore 1 (green) and pore 2 (blue). Visual analysis suggests that pore 1 is more constricted in the ATP state (d). Quantification of the pore size, using snapshots every 25 ns from 750 – 1000 ns: both pores are tighter in ATP bound state (pore 1 means are 1.9 and 2.6 Å², p < 0.0001; pore 2 means are 2.4 and 2.1 Å², p = 0.0061).

Supplementary file 1A. HDX data summary table for ΔHDX = (SecA<sup>AMPPNP</sup>) – (Sec<sup>ADP</sup>). Data is presented in Figure 2b. Mass spectrometry raw data are deposited in the ProteomeXchange Consortium via the PRIDE database.

Supplementary file 1B. HDX data summary table for ΔHDX = (SecA<sup>AMPPNP</sup> + SecYEG) – (Sec<sup>ADP</sup> + SecYEG).

Data is presented in Figure 2c. Mass spectrometry raw data are deposited in the ProteomeXchange Consortium via the PRIDE database.

Supplementary file 2. HDX data summary table for ΔHDX = (SecYEG + SecA<sup>AMPPNP</sup>) – (SecYEG + Sec<sup>ADP</sup>).

Data is presented in Figure 3. Mass spectrometry raw data are deposited in the ProteomeXchange Consortium via the PRIDE database.
Material and methods

Protein preparation

SecYEG and SecA were overproduced and purified as described previously.28

Hydrogen deuterium exchange mass spectrometry (HDX-MS)

The HDX-MS experiments outlined here were carried out using a Synapt G2Si HDMS coupled to an Acquity UPLC M-Class system with HDX and automation (Waters Corporation, Manchester, UK).

To form the SecA-SecYEG complex, SecYEG and SecA were mixed and incubated for 10 min on ice. For experiments investigating SecA (Figure 2), SecA (10 μM) was saturated by SecYEG (15 μM). For experiments investigating SecYEG (Figure 3), SecA (15 μM) was added in excess of SecYEG (10 μM), as described previously.9 5 μl of SecA or the SecA-SecYEG complex was diluted into 95 ml of deuterated buffer (20 mM Tris pH 8, 2 mM MgCl₂, 50 mM KCl, and 0.02 % (w/v) dodecyl-maltoside DDM in D₂O) or with equilibration buffer (20 mM Tris pH 8, 2 mM MgCl₂, 50 mM KCl, and 0.02 % (w/v) DDM in H₂O).

Deuteration was carried out at 25°C. For experiments analysing the Sec complex in the presence of the nucleotides, 1 mM of either the non-hydrolysable AMPPNP or ADP was added to the protein mixture and to equilibrium or deuteration buffer.

The proteins were labelled by incubation in deuteration buffer for 0.25, 1, 5, and 30 minutes to capture short, medium and long exchange times. Deuteration was quenched with 100 μL of quench buffer (0.7 % (v/v) formic acid and 0.1 % (w/v) DDM) at 1 °C and a pH of 2.4.

Protein digestion to peptides was performed at 25°C using an Enzymate online digestion
column (Waters) in 0.1 % (v/v) formic acid at a flow rate of 200 mL/min. Between injection of samples, the pepsin column was washed with cleaning solution (0.8 % (v/v) formic acid, 1.5 M Gu-HCl, and 4 % (v/v) MeOH) recommended by the manufacturer. To reduce peptide carry-over, a blank run was performed between sample runs.

Peptides were trapped using an Acquity BEH C18 1.7 μM VANGUARD pre-column for 3 mins at a flow rate of 200 μL min in a buffer A of 0.1 % (v/v) formic acid ~ pH 2.5. Peptides were eluted into an Acquity UPLC BEH C18 1.7 μM 1.0 x 100 mm analytical column with a linear gradient of 8–40 % (v/v) gradient of acetonitrile with 0.1 % (v/v) formic acid with a flow rate of 40 μL/min. Peptides were then ionized by positive electrospray into a Synapt G2-Si mass spectrometer (Waters). A 20-30 V trap collision energy ramp was utilised to capture the MS data. Electrospray ionization source was operated in a positive ion mode and ion mobility was enabled for all experiments. Leucine Enkephalin was used as a lock mass for mass accuracy correction and iodide was used for mass spectrometry calibration. All deuterium time points were performed in triplicate.

**HDX data evaluation and statistical analysis**

All experiments, including deuterated time points and reference sample controls, were repeated in triplicate. MS data from reference sample controls the complexes were used by the Waters ProteinLynx Global Server 2.5.1 (PLGS) and filtered using DynamX (v. 3.0) to provide sequence identification. The following parameters were used to filter the quality of the peptides: minimum and maximum peptide sequence length of 4 and 25, respectively, minimum intensity of 1000, minimum MS/MS products of 2, minimum products per amino acid of 0.2, and a maximum MH + error threshold of 5 ppm. All spectra generated from the peptides were examined and only peptides with a high quality spectra and a high signal to
noise ratios were used for data analysis. Wood’s plots and confidence intervals were generated using the in-house Deuteros software.\textsuperscript{25}

\textit{In silico analyses of SecA pore dynamics}

Analyses were based on all-atom molecular dynamics simulations of a complex comprising \textit{Bacillus subtilis} SecA, \textit{Geobacillus thermodenitrificans} SecYE and a 76-stretch of pre-protein, built from PDB 5EUL.\textsuperscript{6} The simulations were run in an ATP or ADP-bound state, with full details of their set up described previously.\textsuperscript{9} For the analyses here, only the atoms corresponding to SecA and SecYE were kept (i.e. the solvent, membrane and pre-protein were removed). Snapshots were taken every 25 ns over a range of 750 to 1000 ns.

Two highly conserved pre-protein pores in SecA were identified: pore 1 on the cytoplasmic surface of SecA (Figure 4c: green) and pore 2 close to the SecY binding interface (Figure 4c: blue). Pore 1 consists of residues Ile 222, Ser 224, Gly 326, Arg 327, Arg 328 and Ser 340 (in \textit{B. subtilis} numbering; equivalent to \textit{E. coli} residues Ile 224, Ser 226, Gly 346, Arg 347, Arg 348 and Ser 350). Pore 2 consists of Gln 595, Tyr 599 and Gln 736 on SecA (in \textit{B. subtilis} numbering, \textit{E. coli} equivalent are Gln 644, Tyr 648 and Gln 787), and Ile 243, Tyr 245 and Ala 246 on the functionally-important\textsuperscript{29} C4 loop of SecY (\textit{G. thermodenitrificans} numbering, approximately equivalent to Val 246, Tyr 248 and Ala 249 on \textit{E. coli} SecY).

Note that residues Gly 346, Arg 347, Arg 348 and Ser 350 in pore 1 and Gln 787 and Ala 249 of pore 2 (all in \textit{E. coli} numbering) are shown to be deprotected in the HDX data (see Data Availability).

To quantify the pore size in the different snapshots, cavity cross-sectional area analyses were run using HOLE.\textsuperscript{30} The algorithm was set to start from a position in either pore 1 (between
the α-carbons of residues Ile 222 and Ser 340) or pore 2 (between the α-carbons of residues Tyr 599 and Ile 243). To account for flexibility in the pore position, a region of ~0.9 nm on either side of the starting point was selected for analysis, and the pore defined as the narrowest point in this region. The pore size was quantified based on the average over a 0.35 nm window, to reduce artefacts arising from thermal fluctuations.

Images were made using the PyMOL Molecular Graphics System version 2.1.1, Schrödinger, LLC, and data were plotted and analysed in Prism version 7, GraphPad Software.

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier: PXD013594. SecYEG-SecA (saturated SecYEG from Corey et al., eLife 2019;8:e41803 doi: 10.7554/eLife.41803) data can be accessed using the with the dataset identifier: PXD013594.

Acknowledgment

This work was supported by the Wellcome Trust (109854/Z/15/Z) and a King’s Health Partners R&D Challenge Fund through the MRC, Confidence in Concept (MC_PC_15031) to A.P. This work was funded the BBSRC (BB/N015126/1 to IC; BB/M003604/1 to IC and RAC; BB/I008675/1 to IC and WJA). Extended simulations were run on the ARCHER UK National Supercomputing Service (http://www.archer.ac.uk), provided by HECBioSim, the UK High End Computing Consortium for Biomolecular Simulation (hecbiosim.ac.uk), supported by the EPSRC. EP is the recipient of an Imperial College London, Institute of Chemical Biology EPSRC CDT studentship.
The authors declare no competing interests.

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ΔHDX = (SecA AMPPNP) − (SecA ADP)

ATP binding site

Cl 99%: ±0.44 Da

ΔHDX = (SecA AMPPNP + SecYEG) − (SecA ADP + SecYEG)

Cl 99%: ±0.41 Da
\[ \Delta \text{HDX} = (\text{SecYEG} + \text{SecA}^{\text{AMPNP}}) - (\text{SecYEG} + \text{SecA}^{\text{ADP}}) \]

**SecY**

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**SecE**

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**SecG**

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