

Case Study Template 1

Please complete 3 short case studies (max. 2 pages each). These case studies can be updates of the ones that have already been submitted (if so please note this in the case study title) or new case studies that have been developed in the current reporting period.

1. Title of Case Study: The mechanism of protein translocation across cell membranes
2. Grant Reference Number: <ul style="list-style-type: none">• BBSRC: BB/I006737/1; BB/M003604/I; BB/I008675/1• Wellcome Trust: 104632• ERC: FP7/2007-2013 / ERC grant agreement 32240
3. One sentence summary: Molecular dynamics simulations and experimental studies unpick the mechanism of the transport of proteins across cell membranes by the Sec translocon.
4. One paragraph summary: The essential process of protein secretion is achieved by the ubiquitous Sec machinery. In prokaryotes, the drive for translocation through the SecYEG channel comes primarily from ATP hydrolysis by the cytosolic motor-protein SecA. However, the mechanism through which ATP hydrolysis is coupled to directional movement through SecYEG is unclear. Through a combination of all-atom MD simulations and single molecule FRET and biochemical assays, it is shown that ATP binding by SecA causes opening of the SecY-channel at long range, while substrates at the SecY-channel entrance feed back to regulate nucleotide exchange in SecA. This two-way communication suggests a new 'Brownian ratchet' mechanism, whereby ATP binding and hydrolysis bias the direction of polypeptide diffusion. The model represents a solution to the problem of transporting inherently variable substrates such as polypeptides, and may underlie mechanisms of other motors that translocate proteins and nucleic acids.
5. Key outputs in bullet points: <ul style="list-style-type: none">• A novel mechanism for the process of protein secretion through the bacteria Sec translocon• Training of a PhD student in use of molecular dynamics/analysis of large simulation data• Feeding in to an ongoing antibiotic development collaboration with Karen Dowers in Dundee• Provision of new targets for an ongoing Electron paramagnetic resonance collaboration with Janet Lovett in St Andrews.• Feeding directly into a new BBSRC grant using similar techniques to further examine the Sec translocation process (BB/N015126/1).
6. Main body text What?

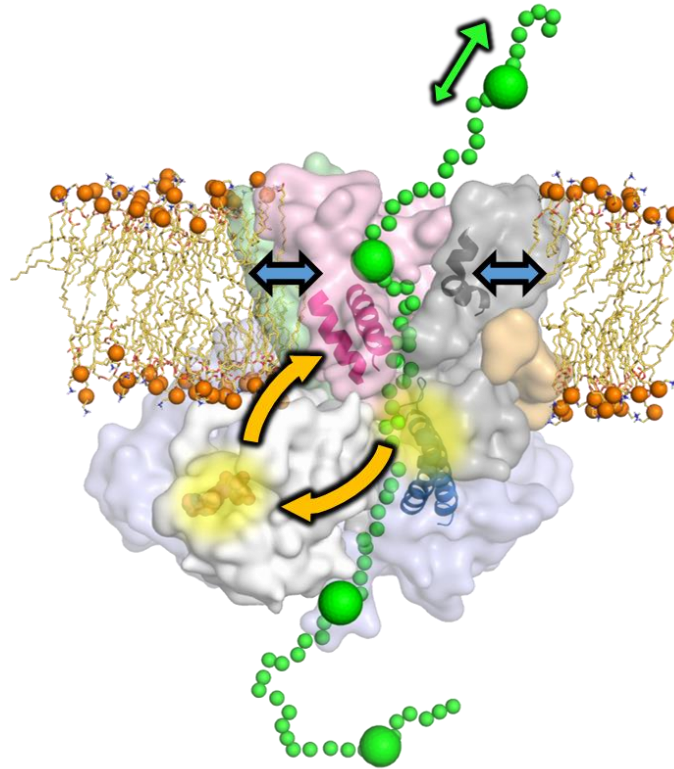
The translocation of proteins across membranes is a fundamental and essential process, achieved in every living cell by the ubiquitous Sec translocon. In bacteria, SecYEG and SecA associate to translocate proteins across the inner membrane post-translationally, powered by ATP and stimulated by the trans-membrane proton motive force (PMF). Despite structural data, the mechanism of how ATP hydrolysis is coupled to protein transport remains unclear.

How?

The entire SecYEG-SecA complex from *Thermotoga maritima* was initially analysed using equilibrium atomistic MD to investigate the role of the SecA ATPase cycle in protein transport. Five independent simulations (~230,000 atoms) of the complex in an ADP or ATP state were simulated, with three (ATP) or four (ADP) of each state simulated to 400-500 ns, and two (ATP) or one (ADP) simulated to 1 μ s. The data were used to determine the conformations of the complex in the different nucleotide states, thus probing the conformational changes associated with the SecA hydrolysis cycle.

The data suggest a coupling of SecA nucleotide occupancy to the opening of the SecY protein-channel, with perturbations within SecY feeding back to SecA to regulate nucleotide exchange. The observations are supported using a FRET pair across the SecY lateral gate, and stopped-flow analyses of the rate of nucleotide exchange with different SecYEG variants. Steered-MD simulations of different sized peptides crossing the SecY channel propose a functional difference between these SecY states, namely that the open state is required to translocate bulky regions of pre-protein. Stopped-flow analyses of SecYEG-SecA crosslinked to peptides demonstrate that nucleotide exchange is promoted by bulky regions of peptide.

These data are consistent with a Brownian motor mechanism; whereby the opening and closing of the channel are synchronised to the hydrolytic cycle of ATP, co-ordinated also with the translocating pre-protein, to set up a ratchet allowing diffusion across the membrane while preventing backsliding.



Why?

Figure legend: Overview of proposed model for ATP-driven protein translocation through SecYEG-SecA complex. In this figure, the protein is shown as surfaces, with SecA coloured in light blue and white, SecE coloured in light orange, SecG coloured in green and the two halves of SecY coloured in pink and grey. The bound nucleotide (ADP or ATP) is shown as orange, red and blue spheres. The surrounding lipid membrane is shown as orange spheres and wheat sticks. Shown as cartoon are the two-helix finger of SecA (blue) and the SecY lateral gate (pink and black). A simplified form of the translocating pre-protein is shown as small green spheres, with difficult-to-translocate regions (i.e. "blocks") shown as larger spheres. In the closed state of the complex (with ADP bound), the pre-protein can diffuse freely back and forth unless a block is present. When this occurs, the two-helix finger of SecA 'senses' the block and triggers the release of ADP (yellow arrow). The subsequent binding of ATP results in an opening of the SecY channel (yellow arrow; blue arrows), resolving the blocked pre-protein and once again permitting free diffusion (green arrow). After a set time, ATP is hydrolysed and the complex reverts to the closed state. If the blockage is still on the inside of the channel, the cycle is repeated. If the blockage is on the outside of the channel, then no mechanism exists to open the channel on the outside so the block has been 'ratcheted' through SecY.

The outcomes from this study hold a threefold importance:

- Sec is conserved across all domains of life, and carries out the bulk of protein translocation in every cell of every organism. Understanding the relatively simple bacterial system may provide insights into the role of Sec in other organisms, including humans
- The ATPase SecA is unique to bacteria, where it is absolutely essential for protein translocation. Cells where SecA is absent or compromised are not viable, making SecA a promising possible target for antibiotic design. The Collinson lab is collaborating with Karen Dowers at the University of Dundee on the design of new antibiotic drugs against SecA, for which the findings from the above study are feeding in.
- The novel nature of this mechanism not only represents a breakthrough in our understanding of Sec-mediated translocation, but may also underlie mechanisms of other

motors that translocate proteins and nucleic acids. As such, the findings are of general interest to the field of molecular motors.

7. Names of key academics and any collaborators: William J. Allen^{1,*}, Robin A. Corey^{1,*}, Peter Oatley^{2,3,*}, Richard B. Sessions¹, Steve A. Baldwin^{2,3}, Sheena E. Radford^{2,4}, Roman Tuma^{2,4,†}, Ian Collinson^{1,†}

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8. Sources of significant sponsorship (if applicable): N/A

9. Who should we contact for more information? Ian.Collinson@bristol.ac.uk

10. Please indicate if you would like this case study to be included on the Consortium's ARCHER web-page. Yes.
