Analysis of the mechanism of protein secretion through the Sec machinery and exploitation as a polypeptide sequencing device

Supervisory team:
Main supervisor: Prof Ian Collinson (University of Bristol)
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Host institution: University of Bristol
CASE partner: Oxford Nanopore Technologies

Project description:
Transport of proteins across membranes is a fundamental biological process essential for protein secretion and organelle biogenesis1,2. This CASE project concerns the bacterial system, wherein protein transport from the cytosol across the inner-membrane is usually achieved when the SecYEG protein-channel complex engages the cytosolic motor ATPase SecA (secretion). Great strides have been made towards understanding the mechanism of protein-translocation: firstly, through the determination of the structures of the protein-channel and the motor components 3,4, and secondly, through the development of accurate and high-resolution assays for protein transport. We have developed such an assay, based on a split luciferase system, for both mitochondrial import and bacterial secretion5,6. The project will continue with the exploitation of this technology, together with a wide range of biochemical and biophysical approaches, towards the determination of the underlying molecular basis for protein transport.

In parallel, your CASE project will be partnered with Oxford Nanopore Technologies (ONT) to explore the prospect of exploiting the channel as a polypeptide sequencer. This would be achieved in the spirit of ONT technology developed for DNA sequencing – by monitoring variable conductance as different nucleotides of a single polymer pass through a pore in the membrane. Currently, peptide sequencing is very challenging, time consuming and expensive, so if this can be simplified, and adapted for biological samples then the implications for analytical biochemistry and cell biology research, diagnostics, forensics etc. would be game changing. Early indications are very promising as we know that positive charges (lysines and particularly arginines) and bulky residues struggle to make it through the channel and slow transport considerably5. Therefore, different residues must have distinct interactions with the SecY-channel and might also elicit a measurable difference in conductance, and thereby generate interpretable signatures for different residues required for sequencing. ONT is a globally successful company with a portfolio of technological innovations. Therefore, the partnership will bring together expertise of the Sec machinery together with sequencing know-how and platforms to create an ideal environment for the success of your project, for training and for experience of academic and biotech environments and teamwork.

REFERENCES 1. Needs et al. Life 11, 432 (2021). 2. Troman & Collinson. Front Microbiol 12, 782900 (2021). 3. Berg et al. Nature 427, 36–44 (2004). 4. Zimmer et al. Nature 455, 936–943 (2008). 5. Allen et al. Elife 11, e77586 (2022). 6. Ford et al. Elife 11, e75426 (2022).

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.