Collaboration reveals a new mechanism for protein secretion

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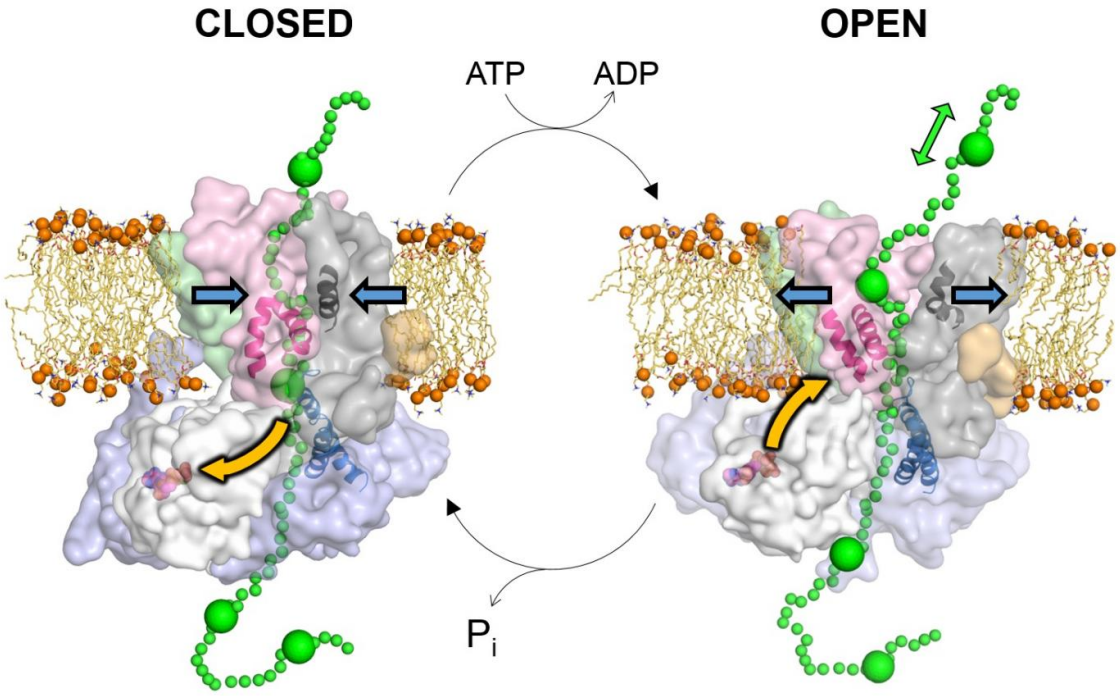

In this figure, the protein is shown as surfaces, with SecA coloured in light blue and white, SecE coloured in light orange, SecG coloured in green and the two halves of SecY coloured in pink and grey. The bound nucleotide (ADP or ATP) is shown as orange, red and blue spheres. The surrounding lipid membrane 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.Left - 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). Right - 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. Credit: None required

A UK research team has discovered that a cell's protective layer acts like a turnstile, allowing proteins to be exported while preventing them from moving back in.

All cells are surrounded by a protective layer - a membrane - which keeps the contents of the cell together and protects it from damage.

However, proteins made inside the cell often need to be exported in order to do their job. For instance, in bacterial adherence, pathogenesis and antibiotic clearance.

The same process in humans is responsible for the secretion of all extracellular proteins, such as collagen, antibodies and insulin.

The researchers, from the Universities of Bristol and Leeds, looked at the specialised 'transport motor' that sits within the membrane, known as 'Sec' (short for secretory). Sec recognises the proteins that need to be exported from the cell via a molecular 'address tag', and pushes them across the membrane. The energy required to do this comes from the cell's universal power source, a molecule called ATP.

Professor Ian Collinson, from the University of Bristol's School of Biochemistry, said: "Previous studies have shown what Sec looks like,

but not how it pushes substrates from one side of the membrane to the other. We used a combination of experimental and computational methods to look at how the different parts of Sec move around as it uses ATP.

"Our reasoning was that it's easier to understand how a motor works by watching it in action rather than just looking at a still picture and trying to then work out how the machine might work."

Co-author Professor Sheena Radford, Director of the Astbury Centre for Structural Molecular Biology at the University of Leeds said: "The work was made possible by the teamwork of the authors, which brought a combination of classical biochemical and biophysical technologies exploring the bulk system of many billions of molecules together with large scale simulations of the protein complex (Bristol), and ground breaking methodologies capable of observing single molecules, pioneered in Leeds by Dr Roman Tuma."

Co-author Dr Roman Tuma, also from the Astbury Centre for Structural Molecular Biology said: "The most popular theory on this at the moment is that Sec grabs hold of part of the protein and pushes it through a gate in the membrane. It then lets go, and goes back to grab and push the next bit.

"However, our study shows that the biggest movement is in the membrane gate itself, which opens and closes. This suggests Sec acts more like a turnstile. Proteins can move freely one way across the membrane - out - but are prevented from moving back in again.

"The new model we present provides a solution to an outstanding problem in the protein transport field, which might be relevant in many other systems that transport proteins and nucleic acids elsewhere in the cell."

The discovery is being used by the team, in collaboration with the Dundee Drug Discovery Unit, towards the development of new drugs that specifically block bacterial secretion without affecting humans. Jamming the secretion process would be harmful to bacteria and their strategies for antibiotic resistance.

The research and collaboration was helped and inspired by our late friend and colleague Professor Steve Baldwin, to whom the paper is dedicated.

More information: William John Allen et al, Two-way communication between SecY and SecA suggests a Brownian ratchet mechanism for protein translocation, *eLife* (2016). DOI: 10.7554/eLife.15598

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