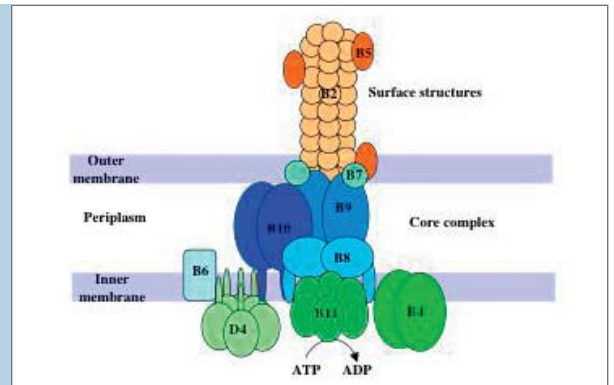


# Secrets of secretion: Characterising type IV secretion systems in Gram-negative bacteria

## SUMMARY

Type IV secretion is an important process in Gram-negative bacteria, allowing bacteria to exchange genetic material (including drug-resistance genes), and pathogenic bacteria to secrete disease-causing molecules. Professor Gabriel Waksman and colleagues have spent a number of years producing 3D structures of the 12 proteins that make up this secretion system. Shortly, they will publish a paper describing the structure of the 'core machinery' – vital for the system's function – and are currently using continued programme grant support to image the entire secretion machinery.



## Background

The type IV secretion system is one of at least six classes of secretion system found in Gram-negative bacteria. It is used by pathogenic bacteria to secrete virulence factors – molecules associated with disease – into host plant or animal cells. This system is also used to transmit plasmids (circular DNA molecules) from one bacterium to another. Plasmids carry antibiotic resistance genes, so type IV secretion is involved in the spread of antibiotic resistance between bacteria.

In the last decade, researchers have begun to explore the structure of the component proteins to understand how these type IV secretion systems work. Gabriel Waksman, Professor of Structural Molecular Biology at University College London and Birkbeck, has been studying the structural biology of these systems since 2000. He and his colleagues use X-ray crystallography, electron microscopy and nuclear magnetic resonance (NMR) to produce images of the secretion 'nanomachines', in order to understand their function.

## Advance

Supported by a Wellcome Trust programme grant, Professor Waksman and colleagues have produced 3D structures of most of the 12 proteins that make up type IV secretion systems. Three of the proteins are ATPases, enzymes that breakdown the chemical ATP to release energy. The remaining components are structural proteins that make up the channel through which virulence factors and plasmids pass.

Gram-negative bacteria are surrounded by two membranes, which the secretion machinery spans. The core machinery – the essential parts of the secretion system that form the channel between the two membranes – consists of insoluble proteins or parts of proteins. The proteins (or parts of proteins) either side of the channel (which face into or out of the bacterial cell) are soluble.

Professor Waksman and colleagues have produced images of the soluble proteins (and parts of proteins) that make up the type IV secretion machinery. Imaging the insoluble proteins that make up the core machinery is more difficult because they exist in a cell membrane environment, while the purification of proteins required for imaging must be performed in a water-rich environment.

However, in an upcoming research paper, Professor Waksman's team will be the first to publish details of the core machinery. This core spans the two membranes and forms the channel through which secreted proteins or DNA must pass. A renewed programme grant from the Trust, which started in April 2008, is being used to provide additional views of the system and catch the system in the act of translocating DNA or a secreted protein.

## How it's making a difference

Professor Waksman and colleagues have published a number of papers in high-impact journals. Their work generates protein structures, which are openly available through the online Research Collaboratory for Structural Bioinformatics Protein Data Bank ([www.pdb.org](http://www.pdb.org)). These structures help to explain the molecular mechanisms used by secretion nanomachines to export toxins and virulence factors through the cell envelope of pathogenic bacteria.

The knowledge generated by this research also has potential use in the development of new antibiotics and in creating better tools to manipulate the production of biomedically important proteins.