Hanging by a Thread

At one point during their growth cycle, some bacteria literally hang by a thread from the cell they are about to invade – a thread that could become a target for next-generation antibiotics.

Streptococcus pyogenes, which causes sore throats and tonsillitis as well as severe invasive illnesses such as rheumatic fever, attaches itself to human cells by means of purpose-built protein strands called pili. *Streptococcus* is also popularly known as "flesh-eating bacteria" for its role in necrotising fasciitis, an insidious and highly unpleasant soft tissue infection.

Streptococcal pili are so thin that they were not discovered until 2005 by scientists in Italy, although structures with a similar function were known for other bacteria. Pili are often important for bacterial virulence as well as colonisation of human cells, making them an important target for vaccine development.

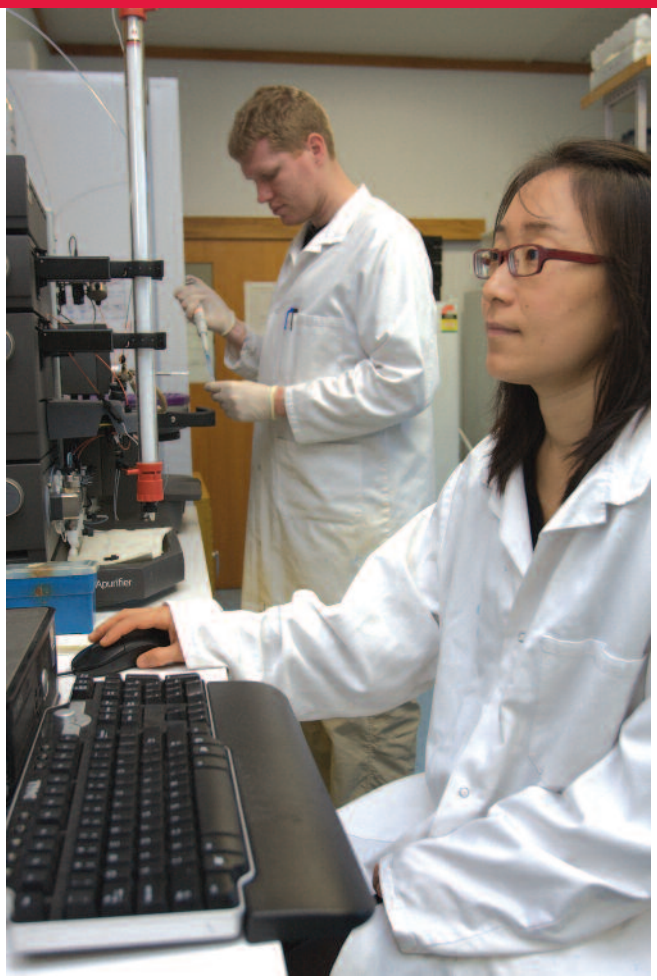
Each streptococcal pilus is 1-4 μm long (about the same length as a typical bacterial cell) and one molecule thick (2–3 nm), but strong enough to withstand extreme physical stress. For example, the structure only disintegrates at temperatures above 75°C, although most biological proteins are notoriously more heat-sensitive.

New Zealand researcher HaeJoo Kang and her colleagues in Professor Ted Baker's group at the University of Auckland are using protein crystallography to obtain detailed three-dimensional structures of the proteins (pilins) that make up the backbone of the pilus structure. Each pilus consists of around 100 pilins piled on top of each other in a head-to-tail arrangement.

HaeJoo says the key to the amazing stability of streptococcal pili is an unusual chemical bond called an isopeptide bond that forms within individual pilin proteins. What makes it unusual is not the bond itself – it's the same kind of bond that joins one amino acid to another to make up a protein backbone chain – but the fact that it occurs between side chains of amino acids.

Synchrotron studies have revealed the detailed structure of the isopeptide bond and its surroundings, which include hydrophobic sections of the pilin protein molecules that protect the active site and create the right environment for the bonds to form. The isopeptide bond structure is highly conserved – it is found in many different strains of the same bacteria. An antibiotic that disrupted its formation would potentially be protective against all these strains, making it a valuable addition to our diminishing stock of effective drugs for common disease organisms.

On a recent trip to the Australian Synchrotron, HaeJoo's colleague Neil Paterson used the PX2 beamline to study the structure of the pilus proteins in *Corynebacterium diphtheriae*, which causes diphtheria. Worldwide vaccination programs have dramatically reduced the incidence of diphtheria, but the disease can still be fatal in as many as one in five cases.



HaeJoo Kang and Neil Paterson (shown here in their Auckland laboratory) are using the Australian Synchrotron to study some unusual bacterial proteins. Photo: University of Auckland

The PX2 beamline is favoured for work with microcrystals and weakly diffracting crystals. Its tiny beam size also caters for crystals such as the diphtheria pilin where the unit cell (the main repeating unit of a crystal structure) has one very long edge; this would otherwise create a diffraction pattern with overlapping spots that are almost impossible to resolve.

"Synchrotrons are definitely the future of protein crystallography," Neil says. "With beamlines like PX2 it takes 10 minutes to get data that would otherwise take 2–3 days, and wouldn't be as good."

"Pilins are promising targets for developing vaccines against a range of major disease-causing bacteria," HaeJoo says. "Synchrotrons are playing a big part in helping us to reveal more of their secrets."

"By studying different bacterial pilins, we may also learn how to introduce isopeptide bonds into other proteins to stabilise them against physical stresses," Neil adds. "This could provide exciting potential for biotechnology applications."

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