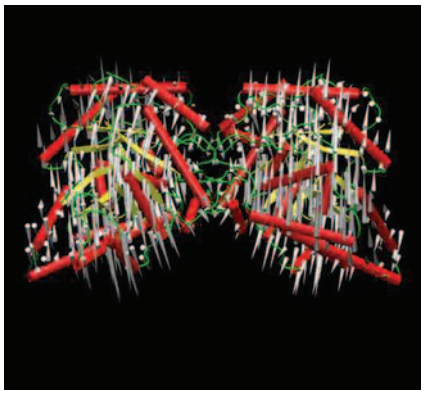
Profile of a Killer Enzyme

A single gram of this microbe's crystalline toxin, evenly dispersed and inhaled, has the power to kill more than one million people. Can synchrotron-based research reveal the microbe's weak spots in time?



Going through the motions: the thermal movement of bacterial enzyme DHDPS depicted using DYNAMITE software authored by Martin Noble.

No, we're not describing the plot of the latest high-tech disaster movie. The powerful botulin toxin is one of the less-endearing characteristics of a bacterium called *Clostridium botulinum*. A potential bioterrorism agent, *C. botulinum* may also be a cause of sudden infant death syndrome.

The good news is that *C. botulinum* is now on Dr Matt Perugini's hit list at the University of Melbourne, along with other high-profile bacterial nasties such as anthrax, drug-resistant *Streptococcus pneumoniae* and golden staph.

Anthrax spores can survive in the soil for decades, waiting to infect animals that graze close to the ground, which they are forced to do in times of persistent drought. Anthrax could potentially kill millions of Australian livestock and it is fatal to people as well. Its use as a bioterrorism agent has already killed five people in the US.

S. pneumoniae is a major cause of lower respiratory infections, which kill more than four million children per year, but 70% of its strains are resistant to antibiotics.

Golden staph (*Staphylococcus aureus*) is a common skin bacterium that causes a wide range of diseases, from minor skin infections to meningitis, pneumonia and septicaemia. Drug-resistant strains such as methicillin-resistant *S. aureus* (MRSA) are a major problem in hospitals because bacterial infections can easily be transferred in the hospital environment and there are few alternatives to methicillin-type antibiotics.

Perugini is using an approach he hopes will "kill four bacteria with one stone". He is examining the fine structure of an enzyme present in all four bacteria with the aim of finding a way to stop it functioning. Dihydrodipicolinate synthase (DHDPS) catalyses a key step in the biosynthesis of lysine, a crucial component of bacterial cell walls. This biosynthetic pathway is absent in humans, making the enzyme a potentially useful target for antibiotics.

Identifying opportunities to block or modify the action of biologically important molecules such as DHDPS is the first stage of structure-based drug design, a drug discovery process that uses synchrotron X-rays to reveal the detailed structures of biological proteins and their interactions.

Perugini and his colleagues used the protein crystallography beamline at the Australian Synchrotron in Melbourne to determine high-resolution, three-dimensional structures for DHDPS from his bacterial targets. Early studies showed that the active form of the enzyme was a tetramer – four individual units joined together by weak chemical bonds. The tetramer is much less wobbly than a single unit, and can therefore function more efficiently.

In MRSA, however, the enzyme is a dimer (two units) rather than a tetramer, with some structural differences that stabilise the dimer and maintain its effectiveness.

The researchers believe that the tetramer, which is found in a wide range of bacterial species, evolved from an ancient primordial monomer via a less-stable dimer. They speculate that the MRSA dimer is a result of divergent evolution (i.e. it evolved independently of the tetramer, but for the same reason).

Perugini believes that the identification of an active DHDPS dimer will aid the development of an antibiotic that works against a wide range of bacteria – as well as providing an interesting insight into the molecular evolution of this essential bacterial enzyme. His team is screening a large number of small molecules to identify those that block the action of DHDPS.

The protein crystallography beamline is the Australian Synchrotron's busiest beamline, accounting for almost half the facility's users. Dr Julian Adams, who heads the synchrotron's protein crystallography team, says that the beamline is mostly used for structure-based drug design targeting cancer, Alzheimer's disease, heart disease, diabetes, influenza and a range of bacterial enzymes. The remaining 30% of user time on the beamline is devoted to structural genomics (large-scale determination of three-dimensional protein structures, typically involving the full set of proteins expressed by an organism or group of organisms) and fundamental studies of biochemical pathways and receptor systems.

Perugini says the high-throughput beamline has revolutionised his research and given him access to new methods for structure determination.

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