ESRF	**Experiment title:** Structure determination of alpha toxin from the proteolytic strain (NCTC-8237) of *Clostridium perfringens*	**Experiment number:** LS1672
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Report:

Clostridium perfringens is an anaerobic gram-positive, spore forming non-motile rod-shaped organism that commonly resides in soil and the intestines of humans and other animals. The bacterium produces at least 12 extracellular toxins, of which α, β, ε and *iota*-are considered to be major toxins and cause a range of diseases in humans and domestic animals. Alpha-toxin (α-toxin) is the key virulence determinant for gas-gangrene in humans. This toxin is also implicated in the pathogenesis of several other diseases in humans and other animals.

Over the years we have determined several structures of this protein from three different bacterial strains (NCTC-8237, CER89L43 and a divergent strain isolated from an infected swan). Of these strains NCTC-8237 is proteolyticly sensitive and the crystals of α-toxin from this strain have never diffracted beyond 3Å resolution even at SRS station 9.6 or Photon Factory, Japan, whereas those from the other two strains, which are proteolyticly resistant, always diffracted 2Å resolution or better. The *C. perfringens* α-toxin is a zinc metallophospholipase C and the 3D-structure is composed of two domains, the catalytic N-terminal domain is α-helical and carries the site for phospholipid hydrolysis, while the C-terminal doamin is an 8-stranded β-sandwich and has been implicated in membrane binding. A flexible linker connects these two domains. The overall fold of this enzyme is very similar in the different crystal forms, though the hinge angle between the domains varies by 4-6° among the various structures. However, the conformational variability in two different loops

esulted in an active site accessible to substrates in one form, called 'the open form' and an active site obscured by a helix and in therefore not accessible to the substrate in the other called 'the closed form'.

During this allocation period we have collected a 2.5Å native data-set, from a crystal of NCTC-8237 strain and grown from ammonium sulphate at pH 7.8 which is the first time crystals of this strain have diffracted so well. In past, this crystal never diffracted beyond 3Å resolution, and complete tracing of the chain was not been possible from those data sets.

The refinement of the structure is now in progress. We hope to understand the mechanism of opening and closing of the active site from this high resolution structure when it is compared with previously solved structures of α-toxin.