ESRF	**Experiment title:** Structure determination of Epsilon toxin 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. Of these toxins, the epsilon toxin (ε-toxin) causes the pulpy kidney disesase, also known as overeating disease, in pigs, sheep, goats and cattle.
Overeating disease causes a significant number of deaths in these species. The disease is the result of bacteria together with undigested food leaving the stomac and entering the intestine where they can produce epsilon toxin, as a prototoxin. The mature toxin is produced by cleavage of an N-terminal peptide. The ε-toxin (prototoxin) is a 31.5kDa protein, which is cleaved to form the mature toxin of 29.5kDa and is composed of 311 amino acids. Very little is known about the mechanism of toxin, except that the toxin is known to interact with an unidentified cell-surface receptor and to bind to specific cell types.
In order to understand the mechanism of action of this toxin we chose to determine its 3D-structure by X-ray crystallography using SIR/MIR/MAD, as there is no significant sequence homology with any other determined 3D-structure.
The protein was isolated from *C. perfringens* and we were able to grow diffraction quality crystals in three different conditions. There is a problem with this protein is that, crystals are growing in identical conditions and which are, from their morphology and cell dimensions

apparently identical, actually exhibit range of crystallographic and non-crystallographic symmetries. We have collected several data sets, both from native crystals and from different heavy atom soaked crystals, with the aim of solving the 3D-structure. Unfortunately, we have not yet found a derivative with sufficient phasing power to solve the phase problem and, since the protein was purified from the natural bacterium no Se-Met substituted protein is available.

During this allocation period we have collected three data sets. All of these were heavy atom soaks, one Ethyl mercury chloride (EMC), second one was dipotassium platinum tetracyanide [$K_2Pt(CN)_4$] and the third one was from mercury chloride [$Hg(Cl)_2$]. All these reagents had previously been tested on the home source but only to low resolution. Patterson maps from the home source were promising and so it was thought that higher resolution datasets would produce good phasing statistics. The datasets were intergrated using MOSFLM and scaled with the CCP4 program SCALA, further processing was also done using the CCP4 package. The two datasets collected, although of good quality, failed to scale with native datasets and so the resulting patterson maps were uninterpretable

	$K_2Pt(CN)_4$	EMC	$Hg(Cl)_2$
Diffraction Limit (Å)	2.8	2.6	3.0
Rmerge (%)	5.5	6.5	9.1
I/sd	22.3	19.5	16.7
Comp (%)	97.5	99.3	100
Mult (%)	4.6	3.4	4.2