ESRF	**Experiment title:** Structure determination of Epsilon toxin of *Clostridium perfringens*	**Experiment number**: LS1810
Beamline: ID14 EH1	**Date of experiment**: from: 01.02.01 to: 02.2.01	**Date of report**: 27.02.01
Shifts: 1	**Local contact(s)**: Dr. Stephanie Arzt	*Received at ESRF:*

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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 stomach 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.

Previous attempts to solve the stucture using wild-type protein isolated from *C. perfringens* have proved unsuccessful. The reason being that crystals growing in identical conditions which are, from their morphology and cell dimensions apparently identical, actually exhibit a

range of crystallographic and non-crystallographic symmetries. A recombinant mutant of the protein has, therefore, been produced with the aim of producing seleno-methionine labelled protein. This is the first native data set to be collected using recombinant protein.

	Native
Space group	P321
Diffraction Limit (Å)	2.5
Rmerge (%)	6.2
I/sd	12.3
Comp (%)	96.5
Mult (%)	2.0