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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structure determination of *Clostridium absonum* phospholipase C.	**Experiment number**: LS1941
Beamline: ID14 EH1	**Date of experiment**: from: 21.04.01 to: 24.4.01	**Date of report**: 20.08.01
Shifts: 1.5hrs	**Local contact(s)**: Dr. Hassan Belrhali	*Received at ESRF:*

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Report:

The genus *Clostridium*, traditionally defined as containing gram-positive, anaerobic, spore forming non-motile rod-shaped bacteria, constitutes a phylogenetically incoherent genus and are widespread in the environment. They participate to the degradation of organic compounds. Some of them, such as *Clostridium perfringens*, *Clostridium novyi*, *Clostridium bifermentans*, *Clostridium absonum* etc. produce potent toxins and are pathogenic to human and animals. They are mostly associated with two types pathology according to the mode of acquisition, gastrointestinal diseases and gangrene. These toxins are also implicated in the pathogenesis of several other diseases in humans and other animals.

As an on going research programme we are studying the structure and function of alpha-toxin from different *clostridial* specieces. 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 a swan). 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 4-6º among the various structures. However, the conformational variability in two different loops resulted 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’.

Recently the production of phospholipase C by C. absonum has been reported and we cloned, expressed and purified this enzyme from *E. coli* to homogeniety. We were also successful to produce diffraction quality crystals in two different crystal forms.

During this allocation period we have collected a 2.5Å data-set, from a crystal of form-I. The symmetry of the crystals are orthorhombic with four molecules in the asymmetric unit. The structure determination is now in progress.

Summary of the data sets:

Space group	$P2_12_12$
Cell parameters	193.6 Å, 92.96 Å and 92.69 Å
Diffraction Limit (Å)	2.5
Rmerge (%)	6.2% (19.5% in the 2.64-2.5 Å shell)
I/sd	8.0 (2.1)
Comp (%)	96.1% (90.0%)
Mult (%)	2.6