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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: Structural studies of carbohydrate-modifying enzymes**	**Experiment number**: LS-2029
Beamline:	**Date of experiment**: from: 8/2/2002 to: 9/2/2002	**Date of report**: 26/08/2002
Shifts:	**Local contact(s)**: Elena Micossi	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sine Larsen

Leila Lo Leggio*

Pernille Harris*

Report:

Between the time of our earlier application and allocated beam time, the structure of *E. coli* glycogen branching enzyme was reported by another group. This project has therefore been abandoned.

At the time of beam-time allocation, no crystals of pectinases were available for measurement. Therefore we attempted to collect data for a family 9 glycoside hydrolase from the thermophilic organism *Alicyclobacillus acidocaldarius* [1], which crystallizes as extremely thin plates and for which diffraction has only been observed to 6-8 A resolution at conventional synchrotron beamlines. After trial of at least ten crystals, one was found that gave weak but ordered diffraction in all directions. A data set was collected which allowed determination of the space group ($P2_12_12$) and cell (a=85.0, b=129.7, and c=48.6). The data set was collected to a maximum resolution of 3 A, with Rsym=0.154 and a completeness of 99.4% [2]. Molecular replacement using the closest homologue for which 3-D structure is available (*C. thermocellum* CelD, PDB ID: 1clc) gave a convincing solution with one molecule in the asymmetric unit. However, refinement has proven problematic because of low resolution and limited identity between model and target (25-30%).

Within the theme of carbohydrate-modifying enzymes, previous data collected at the ESRF by our group has recently proved fruitful. A native data set collected at ID14-4 of *Aspergillus aculeatus* rhamnogalacturonan lyase [3] has been used to solve the structure by MIR to 1.5 Å resolution (McDonough *et al*, in preparation). The structure represents a novel fold with

three-domain all beta structure, in which all domains are likely to participate in the formation of the active site. A data set collected also at ID14-4 of a maltose O-acetyltransferase crystal to 2.15 Å resolution [4] has also been used for final refinement of the structure (Lo Leggio *et al.*, in preparation). The structure presents the expected trimeric left-handed parallel beta helix fold (Fig. 1).

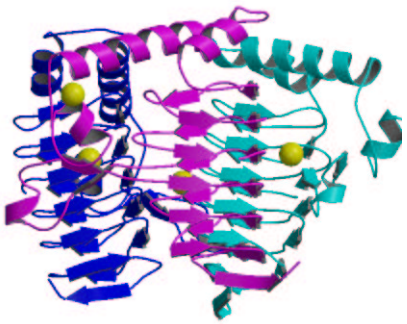

Figure 1 - Structure of maltose O-acetyltransferase

[1] Eckert, K., Zielinski, F., Lo Leggio, L. and Schneider, E. “Gene cloning, sequencing and characterization of a family 9 endoglucanase (CelA) with an unusual pattern of activity from the thermoacidophile *Alicyclobacillus acidocaldarius* ATCC 27009” submitted to *Applied Microbiology and Biotechnology*

[2] Eckert, K., Ernst, H.A., Schneider, E. Larsen, S. and Lo Leggio, L. “Crystallization and preliminary X-ray analysis of *Alicyclobacillus acidocaldarius* endoglucanase CelA” submitted to *Acta Crystallographica Sect. D.*

[3] Kadirvelraj, R., Harris, P., Poulsen, J.-C.N., Kauppinen, S. and Larsen, S. “A stepwise optimization of crystals of rhamnogalacturonan lyase from Aspergillus aculeatus” (2002) *Acta Crystallogr. D58*, 1346-1349

[4] Lo Leggio, L., Dal Degan, F., Poulsen, P., Sørensen, S.Ø., Harlow, K., Harris, P. and Larsen, S. “Crystallization and preliminary X-ray analysis of maltose O-acetyltransferase” (2001) *Acta Crystallogr. D57*, 1915-1918.