EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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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**:
Beamline: ID29	**Date of experiment**: From: 16/4/2003 to: 18/4/2003	**Date of report**: 5/8/2003
Shifts: 6 (shared with MX94)	**Local contact(s)**: Andrew McCarthy	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sine Larsen

Leila Lo Leggio*

Heidi A. Ernst*

Eva Johansson*

Report:

The main projects in the original applications involved the retrieval of phase information by MIR for a family 9 and family 30 glycoside hydrolases, the *Alicyclobacillus acidocaldarius* endoglucanase CelA (Cel9A) (Eckert *et al.*, 2002) and human glucocerebrosidase (GC) (Ginns *et al.*, 1984), respectively.

No diffraction data could be measured for heavy atom derivatized crystals of Cel9A. Only few diffraction quality crystals could be obtained as most crystals are too thin or disordered (Eckert *et al*, 2003) or deteriorate in the presence of heavy atom compounds.

Several data sets for heavy atom derivatives of GC were collected, but in view of the recent publication of the structure by a competing group (Dvir *et al.*, 2003), structure determination has not been further pursued.

Data for two project within this project area not originally described in the application were also collected. Crystals of a xylanase (Fujimoto *et al.*, 2002) variant with altered specificity for small aryl substrate with respect of wild type were soaked in mother liquor containing high concentration of the products cellobiose and xylobiose. Data extending to 2.0 and 2.7 Å resolution, respectively, were collected. Unfortunately in both cases the active site is not

occupied by the product, but by hydrogen phosphate from the mother liquor. Recently, data from crystals of this variant grown under different crystallization conditions and soaked in the presence of cellobiose have been obtained at ID14-4 (as a backup for beamtime allocated to project MX88). These data are currently being analysed.

Data from very small needles of *Cellulomonas fimi* Man26A (Stoll *et al.*, 1999), which are less than 10 µm in the smaller dimension, were also obtained. These crystals had only recently been obtained, and crystallization conditions had not been optimized. The data is limited, extending only to 3.2 Å and are less than 90% complete because of serious radiation damage. However it encouraged us that optimization could yield crystals of suitable quality for structure determination by Molecular Replacement.

Dvir H., Harel M., McCarthy A.A., Toker L., Silman I., Futerman A.H., Sussman J.L.. "X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease" (2003). *EMBO Rep.*, **4**, 1-6.

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" (2002). *Appl. Microbiol. and Biotechnol.* **60**, 428-436

Eckert, K., Ernst, H.A., Schneider, E. Larsen, S. and Lo Leggio, L. "Crystallization and preliminary X-ray analysis of *Alicyclobacillus acidocaldarius* endoglucanase CelA" (2003). *Acta Crystallographica Sect.* , **59**, 139-141.

Fujimoto Z., Kuno A., Kaneko S., Kobayashi H., Kusakabe I, Mizuno H. "Crystal structures of the sugar complexes of Streptomyces olivaceoviridis E-86 xylanase: sugar binding structure of the family 13 carbohydrate binding module" (2002). *J Mol Biol.* **316**, 65-78

Ginns, E.I., Choudary, P.V., Martin, B.M., Winfield, S., Stubblefield, B., Mayor, J., Merkle-Lehman, D., Murray, G.J., Bowers, L.A. and Barranger, J.A. "Isolation of cDNA clones for human beta-glucocerebrosidase using the lambda gt11 expression system" (1984). *Biochem. Biophys. Res. Commum.*, **123**, 574-580.

Stoll D., Stålbrand H., Warren R.A. "Mannan-degrading enzymes from *Cellulomonas fimi*" (1999) *Appl. Environ. Microbiol.* **65**, 2598-605.