EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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**:
Beamline: ID29	**Date of experiment**: From: 5/5/2004 to: 6/5/2004	**Date of report**: 29/8/2005
Shifts: 3	**Local contact(s)**: Didier Nurizzo	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sine Larsen

Leila Lo Leggio*

Heidi A. Ernst*

Eva Johansson*

Report:

A data set for a Se-Met derivative of a MalA crystal diffracting to 2.5 Å resolution was collected, but was only complete to 4.5 Å resolution due to a large cell ($P2_1$ a= 322 Å, b=158 Å, c= 321 Å, β= 119 deg.) coupled with high mosaicity. Crystals of unsubstituted MalA were also tested but only diffracted to 4 Å.

Data leading to structure determination of the β-1,4-mannanase from *C. fimi* were also collected. The abstract from an article in press in *Biochemistry* is enclosed. Data for the mannotriose complex were collected at MAX-LAB.

The structure and characterization of a modular endo-β-1,4-mannanase from Cellulomonas fimi.

Jérôme Le Nours, Lars Anderson, Dominik Stoll, Henrik Stålbrand, Leila Lo Leggio

The endo-β-1,4-mannanase from the soil bacterium *Cellulomonas fimi* is a modular plant cell wall degrading enzyme involved in the hydrolysis of the backbone of mannan, one of the most abundant polysaccharides of the hemicellulosic network in the plant cell wall. The crystal structure of a recombinant truncated endo β-

1,4-mannanase from *Cellulomonas fimi* (CfMan26A-50K) was determined by X-ray crystallography to 2.25 Å resolution using the molecular replacement technique. The overall structure of the enzyme consists of a core $(\beta/\alpha)_8$-barrel catalytic module characteristic of clan GH-A, connected via a linker to an immunoglobulin-like module of unknown function. A complex with the oligosaccharide mannotriose to 2.9 Å resolution has also been obtained. Both the native structure and the complex show a cacodylate ion bound at the -1 subsite, while subsites -2, -3, and -4 are occupied by mannotriose in the complex. Enzyme kinetic analysis and the analysis of hydrolysis products from manno-oligosaccharides and mannopentitol suggest five important active-site cleft subsites. CfMan26A-50K has a high affinity -3 subsite with Phe325 as an aromatic platform, which explains the mannose releasing property of the enzyme. Structural differences with the homologous *Cellvibrio japonicus* β-1,4-mannanase (CjMan26A) at the -2 and -3 subsites may explain the poor performance of CfMan26A mutants as "glycosynthases".