ESRF	**Experiment title:** Fungal cellulases	**Experiment number**: LS-1205
Beamline: ID14-4	**Date of experiment**: from: 20 Nov 1998 to: 23 Nov 1998	**Date of report**: 1 March 1999
Shifts: 2	**Local contact(s)**: Sean McSweeney	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Jerry Ståhlberg, Swedish Univ. Agric. Sciences, Jerry. Stahlberg@molbio.slu.se
T. Alwyn Jones, Uppsala University, alwyn@xray.bmc.uu.se
Sherry L. Mowbray, Swedish Univ. Agric. Sciences, mowbray@alpha2.bmc.uu.se
* Mark Harris, Uppsala University, markh@alpha2.bmc.uu.se
* Mats Sandgren, Uppsala University, mats@alpha2.bmc.uu.se
* Alexander Cameron, Uppsala University, alex@alpha2.bmc.uu.se

Report:

This was our first visit and our first opportunity to use the beamlines at ESRF. The two days allocated were shared with two other projects from our department. Due to machine problems only about 4 shifts (of 6 scheduled) could be used for data collection and of these about 2 were used for this project. Crystals that had been tested for good diffraction on our local rotating anode x-ray source were brought frozen to ESRF for data collection. For some of the crystals, however, the strong diffraction at ID14-4 revealed defects that were not evident from the diffraction pattern obtained at home. Furthermore the data collection was so fast that all time was needed for mounting crystals, backing up data and document parameters for the data collection, and there was no time for indexing and data processing. Therefore some of the collected datasets later turned out not to be useful for structure determination, but they anyhow told to which resolution the different protein crystals may diffract at this beam-line. Several crystals diffracted to very high resolution, but due to how the beam-stop was attached to the instrument the detector could not be set closer than giving roughly 1.4 A resolution at the edge. Higher resolution could have been obtained by raising the detector, but that would have required longer time for data collection and in most cases we chose to optimise the data collection for speed rather than resolution.

Crystals used were cellulases from the filamentous fungus *Trichoderma reesei*, Cellobiohydrolase 1, CBH1 (Cel7a); Cellobiohydrolase 2, CBH2 (Cel6a); Endoglucanase 3, EG3 (Cel12a) and a family 45 endoglucanase, EG5, (Cel45a) isolated from Blue mussel, *Mytilus edulis.*

8 datasets were collected:

1) CBH1 wildtype, co-crystallised with a tetrasaccharide inhibitor, GG-S-GG, where the oxygen at the susceptible glycosidic bond had been replaced by a sulfur. < 1.4 A. Structure refinement underway.
2) CBH1 wildtype, soaked with a trisaccharide inhibitor, where the Glc residue at the non-reducing end had been replaced by a glucono-lactam-oxime moiety (1+2 lactam oxime). < 1.4 A. Disordered and probably not useful.
3) CBH1 delta245-252 mutant, where a loop covering the cleavage site had been deleted. < 1.4 A. Structure refinement underway.
4) CBH1 E217Q mutant (catalytically deficient), soaked with a methyl-umbelliferyl-pentasaccharide where the glucose residue at the non-reducing end was linked by an alpha-1,4 bond instead of the normal beta-1,4 found in cellulose, in order to fix the position and prevent sliding of the oligosaccharide in the 10 Glc long substrate-binding tunnel of the enzyme. < 1.4 A. Structure refinement underway.
5) CBH2 wildtype, soaked with a trisaccharide inhibitor, where the glc residue in the middle had been replaced by a glucono-lactam-oxime moiety (2+1 lactam oxime). 1.6 A. Structure refinement underway.
6) EG3 wildtype, soaked with cellotetraose. 1.8 A. Structure refinement underway.
7) EG3 wildtype, soaked with cellopentaose. 1.8 A. Disordered and probably not useful.
8) EG5 wildtype, co-crystallised with cellobiono-imidazole. 1.5 A. Structure refinement underway.

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