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:** Crystallographic studies of lactate oxidase from Aerococcus viridans	**Experiment number**: MX310
Beamline:	**Date of experiment**: from: 16.09.04 to: 17.09.04 and 06.12.04 to: 07.12.04	**Date of report**: 15.02.05
Shifts:	**Local contact(s)**: Dominique Bourgeois (1st period)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Edward Hough, Arne Smalås, Bjørn Olav Brandsdal*, Ronny Helland*, Ellen Wang*

University of Tromsø

Department of Chemistry

N-9037 Tromsø

Norway

Report:

Background

The protein crystallography laboratory at the University of Tromsø has been regular user of ESRF for many years. Over the years this has resulted in more than 40 publications and a considerable number of PhDs and MScs. The Norwegian Structural Biology Centre (NORSTRUCT) is administrated by the Department of Chemistry at the University of Tromsø, and was established in 2002 through a national initiative in functional genomics in Norway. The aim of this initiative is the establishment of a structural biology centre of high international standard for determination and analysis of the 3D-structures of biologically active macromolecules. In addition to taking part in projects nationwide as an external collaborator, NORSTRUCT has been given the opportunity to initiate and develop internal projects at the centre. Our involvement in external projects range from consultancy to full scale structure determination and structure-function analysis, including hosting project workers for training and providing access to facilities.

Internal projects at NORSTRUCT focus on proteins expressed by the fish pathogenic bacteria *Vibrio salmonicida* and enzymes involved in the defence systems of Atlantic cod and Atlantic salmon, and with a structural genomics approach to virulence factors and defence molecules of the model organisms. "*Structural genomics studies of Vibrio salmonicida*", is one part of a more comprehensive project on this psychrophilic and pathogenic bacteria, also including genome sequencing and cellular/functional studies. The structural part of the project is divided into sub-groups based on functional aspects of the proteins. A) "*Structure-function relation studies of proteins involved in oxidative stress*", B) "*Structure-function relation studies of nucleases", C) "Structure-function studies of DNA repair proteins*", D) "*Structure-function studies of hypothetical proteins*", and E) "*Structure determination of virulence factors expressed by V. salmonicida*".

External projects originate both in the academic society in Norway and in the biotecnology industry, and include nucleases and DNA binding proteins, phosphatases, isocitrate dehydrogenases and several other proteins of academic and commercial interest. The majority of the projects are the subject of structure-function-relation studies, where one seeks to increase the the knowledge about the relationship between structure and biophysical properties such as specificity, efficiency and stability. Succeding structure determination several of the proteins will be the target of redesign of one or more such properties.

Results:
Six shifts were allocated for three projects:

MX308	Structure study of DNase from shrimp
MX309	Structure studies of three glycosylated proteins from Atlantic salmon (Salmo salar)
MX310	Crystallographic studies of lactate oxidase from Aerococcus viridans

Data were collected 16-17.09.04 and 06-07.12.04.

Structure studies of DNase from shrimp
Recombinant DNase from shrimp is produced in Pichia Pastoris. New crystals of DNase were obtained from the same conditions as previously reported (MX253; 14 – 16% PEG 6K, 0.1 M citrate buffer at pH 5 and 2mM WO_4^{2-}) but from microbatch experiments instead of hanging drops. The crystals were still thin plates of approximate size ca 0.5 x 0.2 x 0.01 mm^3. The crystals did not diffract as well as in the previous experiment (2.6Å instead of 2.2Å). The crystal morphology appear to have changed and the space group was monoclinic P21 with cell parameters 49.25 x 51.90 x 84.53 mm3 and $\beta = 96.50$. Apparently the length of the c-axis is half of what was observed previously, and the β-angle had changed.

One MAD data set with detector edge at 2.2Å was collected at the W L-III edge at the peak, inflection point and at the remote, and one SAD data set was collected on a different crystal.
The first scan of the MAD data gave R_{sym} of ca 10%, completeness of ca 99, I/σI of ca 6 and anomalous completeness of 90%. The statistics gets slightly worse for the two other scans. The SAD data gave R_{sym} of ca 13, completeness of ca 98%, I/σI of ca 4, anonomalous completeness of ca 96% and multiplicity of ca 8. The anomalous signals in both datasets (according to xprep) were at best weak, and phasing has not been successful yet.

Structure studies of three glycosylated proteins from Atlantic salmon (Salmo salar)
The proteins were purified from salmon liver. The crystals of protein A grow from ca 20% PEG 2K and 0.1 acetate buffer at pH 4.5. The crystals are of approximate size 0.5 x 0.5 x 0.2 mm^3, and diffract to ca 2 – 2.5 Å at ESRF. The protein is co-crystallized with heavy atoms such as W, Yb and Hg.

Crystals cocrystallized from all three heavy atoms were tested. The best data was collected at the L-III edge of Yb. The crystal diffracted to about 2.8Å. Initial indexing suggested the space group to be P21 with cell parameters of ca 81 x 164 x 81 mm^3 and $\beta=108$. However, recent reprocessing of the data suggest that the space group is C2221 with cell parameters of 94 x 132 x 164 mm^3, and anomalous signals is found in xprep. The data is now subjected to further analysis.

Crystallographic studies of lactate oxidase from Aerococcus viridans
Several crystals were tested the first period and the best crystals diffracted to about 2.5 – 3 Å. The diffraction patterns were, however, of such quality that the crystals could not be indexed.

This was the only protein collected on the second period, and several data sets, both at cryogenic temperature and room temperature, were collected. The data have not been processet though, because the PhD student responsible for this has been away on leave of absence for two months.

Other
Crystals of alcaline phosphatase (TAB5) from the Antarctic strain TAB5 were tested. The crystals were thin sheets that diffracted to ca 2.3 Å in one direction and ca 3 on the other. The data were not of such quality that the structure could be solved by molecular replacement, and the anomalous signal was weak. Better data has been collected in experiment MX372

MopE is a protein involved in copper haemostasis. Crystals had been obtained from 2.0 M ammunium sulfate and 0.1 M acetate buffer at pH 4.6. Native data were collected to 2.7Å. The space group was C2 with cell parameters of ca 105.41 x 101.63 x 38.77 mm^3 and $\beta = 101.76$. R_{sym} was ca 13%, $I/\sigma I$ ca 3.5 and ca 99% completeness. More data was collected in experiment MX368