EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 Chlamysin from Chlamys (Chlamys islandica)	**Experiment number**: MX253
Beamline:	**Date of experiment**: from: 08.05.04 to: 10.05.04	**Date of report**: 15.02.05
Shifts:	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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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 four projects:

MX253	Structural Studies of Chlamysin from Chlamys (Chlamys islandica)
MX233	Structure study of DNase from shrimp
MX234	Structure studies of three glycosylated proteins from Atlantic salmon (Salmo salar)
MX235	Structure-function studies of a nuclease from the cold adapted Vibrio salmonicida

Structure studies of DNase from shrimp

Recombinant DNase from shrimp is produced in Pichia Pastoris. The crystals of DNase are obtained from 14 – 16% PEG 6K and 0.1 M citrate buffer at pH 5, and are thin plates of approximate size ca 0.5 x 0.2 x 0.01 mm^3. The crystals diffract to ca 2 – 2.5 Å at ESRF.

The crystals for the current experiment were obtained with 2mM WO_4^{2-} present in the crystallization reservoir, and they were back soaked before cryo mounting.

MAD data to 2.2 Å were collected at the peak, inflection point and at the remote. Crystals diffracted anisotropically, the reflections were elongated, and it was difficult to determine whether the crystals were of orthorhombic or monoclinic space group. Data collected later suggested that the space group should be P21212 and with cell parameters of 44 x 47 x 184 $Å^3$.

Reprocessing of the data gives R_{sym} of ca 10%, completeness of ca 99 to 95% for the three data sets, I/σI of ca 5 – 6 and anomalous completeness of 96 – 87%. The anomalous signal is very weak, and phasing has not been successful yet.

A high resolution data set (1.8Å) was also collected, but the data were not complete due to too many overlaps.

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 the best crystals diffract to ca 2 – 2.5 Å at ESRF. The protein is co-crystallized with heavy atoms such as W, Yb and Hg. The crystalswere difficult to index and could be P21, C2 or C222

Several crystals were tested and two data sets were collected, one on a potential WO_4^{2-} derivative, and one on a potential Yb derivative. The P21 cell is 81 x 164 x 81 mm^3 and β=108, and anomalous signal is present (according to xprep) for the Yb-derivative, but phasing has not been successful yet. The scaling statistics is very similar for P21 and C2 with R_{sym} of ca 8 %, completeness of ca 93%, I/σI of 7.6 and anomalous completeness of ca 70%.

Structural Studies of Chlamysin from Chlamys (Chlamys islandica)

No diffracting crystals were found

Structure-function studies of a nuclease from the cold adapted Vibrio salmonicida

No diffracting crystals were found

Other

Crystals of alcaline phosphatase (TAB5) from the Antarctic strain TAB5 were tested. Alkaline phosphatases are widely used in molecular biology for the removal of 5` phosphate groups from DNA and RNA.

TAB5 is a zinc protein and MAD data was collected at the zinc edge on the peak, inflection point and the remote. The crystals were thin plates and diffracted to maximum 2.3Å. One of the cell axis tend to be parallell to the X-ray beam, and complete data was impossible to acheive. The data collected at the peak gave R_{sym} of about 15%, I/σI of about 2.7, completeness of about 69% and anomalous completeness of 43%. The statistics of the other scans were slightly worse. The anomalous signal was very weak and phasig did not succeed.