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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:** Tromsø Structural Biology Centre - application for block allocation of beamtime	Experiment number:**01-02-726**
Beamline: BM01A	**Date of experiment**: from: 15.02.06 to: 19.02.06	**Date of report**: 12.05.06
Shifts: 9	**Local contact(s)**: Dr. Philip PATTISON	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Background

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.

Data collection
Experiment 01-02-726 was allocated 9 shifts in February 2006. Crystals of six different proteins, both in native form and in complex, were tested for diffraction. Eight of the crystals were found to be suitable for data collection:

1. MopE from *Methylococcus capsulatus* (1 set)
2. Superoxide dismutase from *Vibrio salmonicida* (FeSOD B, 6 sets)
3. Endonuclease I from *Vibrio salmonicida* (1 set)

Results
Structure of MopE from Methylococcus capsulatus
Copper is an essential micronutrient for all living organisms but is toxic at elevated levels. It is therefore important for the cell to keep a tight control on the level of copper ions. MopE is a protein that is involved in this process, and it has been found to bind both copper and calcium.
MopE can easily be crystallized from ammonium sulfate and Tris buffer using the hanging drop method. The morphology and cell parameters of MopE crystals change depending on the metal ion added to the crystallization reservoir. The structure has recently been solved to 1.8 Å by SAD phasing using a Hg derivative. The aim of this experiment was to collect data on the native protein.

Data was collected on a crystal with no metal added to the crystallization reservoir (native data). The best crystal diffracted to 1.35 Å and belong to the spacegroup I222 with cell parameters of 72.99 x 88.56 x 101.43 $Å^3$. Data were collected over 250 frames with oscillation of 0.5° and exposure of 50 sec. The structure has been solved by molecular replacement (fig below) and refinement and model building is completed. Preparation of a manuscript will be initiated shortly.

Endonuclease I from V. salmonicida (VsEndA)
Vibrio species contain endonucleases in the extracellular space. The exact function of the endonucleases is not always clear, but is probably to provide nucleic acids for the cell. It is also speculated whether the endonucleases can help the pathogen bacteria through the mucus layers of the host by degrading the nucleic acids in the mucus, and hence, decreasing the mucus viscosity. Endonucleases from *Vibrio salmonicida* and *Vibrio cholerae* are used as model systems for studying environmental adaptation of microorganisms, and the focus is adaption to cold and adaptation to salt.
The structures of native endonucleases from both *Vibrio salmonicida* and *Vibrio cholerae* (experiments MX-235/419 and 01-02-693) have recently been solved. In order to futher understand the catalytic mechanism of the enzyme structures of the two endonucleases in complex with DNA is required.

Crystals of an inactive *Vs*EndA mutant co-crystallized with double stranded DNA were obtained from 25% PEG 4000 at pH 6.0. The crystal diffracted to 2.15 Å, and belonged to space group $P2_12_12_1$. Data was collected over 220 frames with an oscillation of 0.5° and exposure of 120 sec. The structure of the protein was solved by molecular replacement. The enzyme had no substrate bound and the structure was identical to the native protein. Therefore, further refinement and analysis was abandoned.

A manuscript describing the structure of *Vibrio cholerae* endonuclease has been submitted to Acta D.

Structure of Superoxide dismutase (FeSOD B).
FeSOD B is part of a metalloenzyme family that catalyzes the dismutation of superoxide anion radicals into molecular oxygen and hydrogen peroxide. The enzyme is used as a model system for studying the molecular basis of cold adaptation.
FeSOD B crystallizes from several different conditions and at different pH. Hydroxide inhibits the enzyme at high pH, and is therefore considered a substrate analogue of FeSOD B. The aim is therefore to collect data on crystals grown at different pH in order to possibly identify structural differences upon substrate binding.

Six data sets were collexted on crystals grown at pH 4.5, 7.5 and 8.5. The crystals diffracted between 1.7 and 2.0 Å and belong to space group $P3_221$with cell parameters of about 70 x 70 x 170 $Å^3$. Data were collected with oscillation from 0.1° to 0.5°, to yield data sets that were about 99% complete. The structures have been solved and refined, and analysis is now in progress.