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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:**01-02-739**
Beamline: BM01A	**Date of experiment**: from: 13.09.06 to: 16.09.06	**Date of report**: 11.05.07
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 01-02-739 was allocated 9 shifts in September 2006. Crystals of four different proteins were tested for diffraction. Five crystals were found to be suitable for data collection:

1. MopE from *Methylococcus capsulatus* co-crystallized with calcium (1 set)
2. Human fibroblast growth factor (1 set)
3. SoxR from *Vibrio salmonicida* (2 sets)
4. Lysozyme (1 set)

In addition, crystals of four other proteins were tested, but the diffraction quality was not sufficient for data collection.

5. Fur from *Vibrio salmonicida*
6. Human spectrin
7. Shrimp Dnase
8. Alcaline fosphatase from a *Vibrio* species

Although some of the crystals appeared to diffract well, indexing was problematic for unknown reasons. We also experienced several times during data collection that there were no connection between the detector and the computer as loads of blank images were recorded.

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.

Calcium is expected to stabilize the protein and prevent auto-proteolytic activity, something which is seen in all other MopE structures solved.
The crystal belong to the orthorhombic space group I222 and 100 frames with oscillation of 1.0° and 20 sec. exposure were collected. The crystal diffracted poorly and could be scaled with reasonable statistics only to about 2.3 Å. Analysis of the structure showed that ths protein was identical to the structures already solved.

Human fibroblast growth factor
The fibroblast growth factors (FGF) are proteins involved in the regulation of a variety of biological processes. By controlling cell proliferation, differentiation function, FGFs are of crucial importance in the development of normal cells. The growth factors are also involved in the maintenance of tissue and healing and repair of wounds. In addition, FGFs have been found to be heavily involved in less favorable processes that ultimately may have pathological consequences.
The acidic fibroblast growth factor (aFGF) possess unusual low stability, and in order to understand the basis and nature of this low stability and thereby learn more about factors influencing protein stability, a range of "stability mutants" of aFGF has been constructed

Data were collected on a triple mutant to 1.6 Å. The crystal belonged to the orthorhombic space group $P2_12_12_1$ with cell parameters of about 34 x 57 x57 $Å^3$. Data were recorded in 100 frames with oscillation of 1.0° and 15 sec. exposure. The structure of the protein was solved by molecular replacement. Analysis of the protein and preparation of a manuscript is in progress.

SoxR from Vibrio salmonicida
SoxR is involved in the regulation/stimulation of oxidative stress genes. In *E.coli* SoxR activates the transcription of *soxS,* which in turn controls the superoxide response regulon. In *V.salmonicida* the *soxS* gene is not present in the genome (similar as in *P.aeruginosa*) and alternative activators must be present. SoxR contains a 2Fe-2S iron-sulfur cluster that may act as a redox sensor system that recognises superoxide. The variable redox state of the Fe-S cluster is employed *in vivo* to modulate the transcriptional activity of soxR in

response to specific types of oxidative stress. Upon reduction of 2Fe-2S cluster, SoxR reversibly loses its transcriptional activity, but retains its DNA binding affinity.

No protein with homologous structure to SoxR is known. The structure of SoxR must therefore be solved by heavy atom methods. Data were collected on two crystals soaked with heavy atoms, one putative mercury derivative and one putative tungsten derivative.
Data were recorded in 360 frames with oscillation of 0.5° and exposure of 45 sec. on the mercury derivative. The crystal diffracted to about 3 Å. Indexing suggested that the crystal could belong to the monoclinic space group C2 or one of the trigonal space groups. Processing of the data was not stable, and unfortunately we have still not been able to establish the right space group.
The tungsten data were recorded on more that 1900 frames with 0.25° oscillation and 60 sec. exposure. The data could be processed in the monoclinic space group C2 with reasonable scaling statistics to 3Å. Weak anomalous signals were observed to about 5 Å, confirming the presence of a heavy atom in the structure. Unfortunately the signals were too weak to phase the structure.

Lysozyme
Data were collected on a lysozyme test crystal in order to compare the statistics of data collected at SNBL with data collected on a Rigaku MSC home source generator. The crystal diffracted to 1.3 Å, but data was difficult to index and processing was impossible.