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:** Tromsø Structural Biology Centre - application for block allocation of beamtime	**Experiment number**: 01-02-693
Beamline: BM01A	**Date of experiment**: from: 17.11.04 to: 22.11.04	**Date of report**: 24.02.05
Shifts: 15	**Local contact(s)**: Mogens CHRISTENSEN	*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 SNBL and ESRF for many years. Over the years this has resulted in more than 50 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 proteins 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 01-02-693 was allocated 15shifts in 2004.

Data were collected on the following proteins:

1. Protein A ((Glycosylated protein from salmon) 1 set)
2. Isocitrate dehydrogenase from *Pyrococcus furiosus* (1 set)
3. Isocitrate dehydrogenase from *Desulfotalea psychrophila* (3 sets)

Crystals of the following proteins were tested:

4. Isocitrate dehydrogenase from *Archaeoglbus fulgidus*
5. RAC GTPase 7 from *Arabidopsis thalina*
6. Catalase from *V. salmonicida*

Several crystals of the proteins mentioned above were tested.

Results

Structure of glycosylated proteins from Atlantic salmon

The crystals of protein A grow from ca 20% PEG 2K and 0.1 M 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 2.5 Å at SNBL. The protein is co-crystallized with heavy atoms such as W, Yb and Hg. The crystals appear to be space group C222 with cell parameters of 93 x 132 x 164 mm^3.
Data was collected on a potential Hg derivative. The cell could be refined, but integration craches, probably do to a too high mosaicity in the crystal.

Structure of isocitrate dehydrogenase from Pyrococcus furiosus (IDH1)

IDH1 can be crystallized from 0.4 M NH_4HPO_4 and 50 mM $MgSO_4$. The crystals are thin plates up to 0.5 x 0.1 x 0.02mm^3, and diffract up to 2.5 Å. The crystals are highly mosaic, but have been identified to belong to the space group C2. The structure has been solved by molecular replacement, and model building and refinement is in progress.

Structure of isocitrate dehydrogenase from Desulfotalea psychrophila (IDH6)

IDH6 can be crystallized from 1.7 M ammonium sulfate, 0.1 M Tris pH 7.4 and 0.1 M Mg_2SO_4. The crystals are thin plates up to 0.4 x 0.5 x 0.05 mm^3 and diffract up to 1.9 Å. The crystals are generally extremely mosaic, and have therefore been difficult to index and integrate. The high mosaicity has also resulted in too many overlaps, and complete data was difficult to obtain. The crystals were for a long time thougt to be of the monoclinic space group C2, but data collected recently has identified the space group to be triclinic. The structure has now been solved by molecular replacement, identifying three molecules in the asymmetric unit. Model building and refinement is in prograss.

Isocitrate dehydrogenase from Archaeoglbus fulgidus (IDH2)

IDH2 can be crystallized from ca 11% PEG 6000, 0.8 M $MgSO_4$ and 0.1 M succinate buffer at pH 4.25. The crystals are thin rods diffracting only to ca 6Å.

RAC GTPase 7 from Arabidopsis thalina (RAC7)

RAC7 can be crystallized from 20% PEG3350 and 0.1 M BisTris pH 6.5. The crystals were extremely small, but diffracted up to 2.2 Å. Unfortunately the crystals died after few images.

Catalase from V. salmonicida

Catalase can be crystallized from 20% PEG 6000 and 0.1 M Hepes pH 7.25. The crystals are thin, hexagonal rods up to 0.5 mm in length. The best crystals diffracted, unfortunately, only to about 8 Å.

Publications 2004

Czapinska, H., Helland, R., Smalås, A.O. and Otlewski, J. (2004) "*Crystal structures of five bovine chymotrypsin complexes with P1 BPTI variants*." *J. Mol. Biol.*, **344**, 1005-1020.

Leiros, H.-K. S., Brandsdal, B.O. Andersen, O.A., Helland, R., Os, V., Otlewski, J., Leiros, I., Willassen, N.P. & Smalås, A.O. (2004) "*Trypsin specificity as elucidated by LIE calculations, X-ray structures, and association constant measurements*". *Protein Science***, 13**, 1056-1070.

Moe, E., Leiros, I., Riise, E.K, Olufsen, M., Lanes, O., Smalås, A. O. & Willassen, N. P. (2004). *"Optimisation of electrostatic surface potential as strategies for cold adaptation of Uracil DNA glycosylase (UNG) from cod (Gadus morhua).*" *J. Mol. Biol.*, **343**, 1221-1230.

Poster and other presentations including SNBL data

Yang N., Steen, I. H, Karlsen, S., Fedøy, A-E. , Glærum, L., Stokke ,R., Madsen, M. S, Andersen, O.A., Raae, A.J, Martinez, A. Birkeland, N.K., Smålås, A.O. (2004) *"Isocitrate dehydrogenases from various organisms: a model system to study structural basis of biological adaptations to extreme temperatures."* 10th International Conference of Crystallization of Biological Macromolecules, 5-11 June, Beijing, China.

Olufssen, M. (2005) "*Increased Molecular Flexibility Plays a Central Role in Cold-Adaptation of Uracil DNA Glycosylase (UDG)."* Norwegian Biochemical Society Contact Meeting, Tromsø, Norway.