



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.

**Experiment title:**

Tromsø Structural Biology Centre - application for block allocation of beamtime

Experiment**number:**

01-02-693

Beamline: BM01A	Date of experiment: from: 06.07.05 to: 09.07.05	Date of report: 17.08.05 <i>Received at ESRF:</i>
Shifts: 15	Local contact(s): Dr. Dmitry CHERNYSHOV	

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 biotechnology 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. Succeeding structure determination several of the proteins will be the target of redesign of one or more such properties.

Data collection

Experiment 01-02-693 has been allocated 15 shifts in 2004 and 9 shifts in 2005. This report concerns data collected 06.07.05 – 09.07.05

Two crystals were tested and data were collected on both:

1. Nuclease *V. cholera* (1 set)
2. Catalase from *V. salmonicida* (1 set)

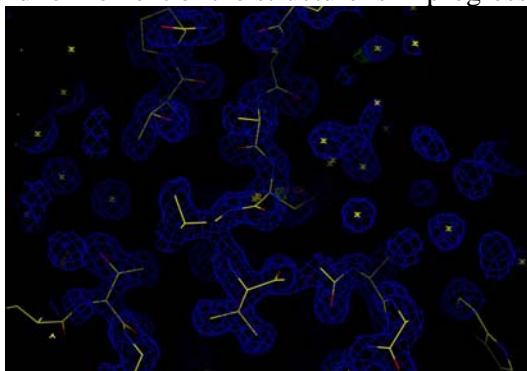
Results

Structure of nuclease from V. cholera

The crystals nuclease grow from ca 25% PEG 4K and acetate buffer. The crystals are of approximate size 0.5 x 0.3 x 0.3 mm³, and the crystals diffract to at least 1.6 Å at SNBL.

Data were collected in MAR 150 x 240 mode over 230 frames using oscillation of 0.5° and exposure of 60 sec. The crystal belonged to space group P212121 with cell parameters of 40.52 x 64.16 x 75.57 Å³. The data scales nicely to 1.6 Å.

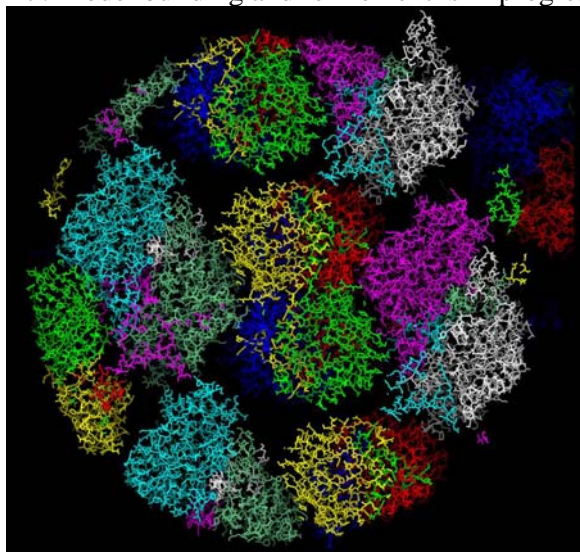
The structure of the nuclease has been solved using molecular replacement, identifying one molecule in the asymmetric unit. Model building and refinement of the structure is in progress.



Structure of catalase from V. salmonicida

Small crystals of catalase grow from PEG 10 and hepes buffer. The crystals are up to 0.1 x 0.4 x 0.4 mm³ and diffract up to 1.8 Å at SNBL.

Initial indexing suggested that the crystal belonged to space group C2 with cell parameters of ca 113 x 160 x 218 mm³, and strategy programs suggested a significant fraction of overlaps. Data were therefore collected in MAR 150 x 345 mode over 180° using 0.25° oscillation and 2 minutes exposure. The crystal was later found to belong to space group P21 with cell parameters of 98.15 x 217.76 x 99.28, $\beta=110.48$. The data scales nicely to 1.96 Å. The structure of catalase has been solved using molecular replacement and with 8 molecules in the asymmetric unit. Model building and refinement is in progress.



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., Smalås, 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.