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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**: 01-02-661
Beamline: BM01A	**Date of experiment**: from: 04.03.04 to: 06.03.04 and 23.06.04 to: 29.06.04	**Date of report**: 24.02.05
Shifts: 9 + 18	**Local contact(s)**: Dr. Philip Pattison	*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 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 01-02-661 was allocated 9 + 18 shifts in 2004.

Data were collected on the following proteins in March:

1. Alcaline phosphatase (2 sets)
2. DNase (1 set)
3. Protein A ((Glycosylated protein from salmon) 1 set)
4. Lactate oxidase (1 set)

Data were collected on the following proteins in June:

5. Alcaline phosphatase (1 set)
6. Lactate oxidase (2 sets; one at 100K and one at 293 K)
7. Protein A ((Glycosylated protein from salmon) 2 sets)
8. Methylpurine DNA glycosylase
9. Isocitrate dehydrogenase from *Pyrococcus furiosus*
10. Isocitrate dehydrogenase from *Desulfotalea psychrophila.*

Several crystals of the proteins mentioned above were generally tested. In addition, several other proteins were tested, but they were unfortunately not of diffraction quality.

The second period (June) was used for testing the CCD detector on proteins. A lot of data were collected. However, complete processing of the data has not been successful yet. After being in contact with Oxford Diffraction and Harry Powell (author of mosflm) we have been able to index the images, but for some reason integration tend to crash, and we have not been able to figure out why. We believe that this could be due to the conversion from one format to another. The person in charge of the data collection has now left the lab and the notes available to us are not clear enough with respect to eg. which pixel size was used. Therefore new data has been collected later on some of the proteins in this run.
The results below refer to the collection in March only.

Results

Alcaline phosphatase ftom Antarctic strain TAB5 (TAB5)

The crystals of TAB5 are very thin sheets that tend to bend in the cryo loops. The crystals tended to diffract anisotropically to ca 2.5 Å in one direction and ca 3 in the other. The reflections were often elongated, resulting in diffraxtion patterns that were difficult to index and integrate. The longest axis was often positioned parallell to the X-ray beam, making it difficult find the correct length of the axis and to get complete data. Results obtained so far indicate that the crystals belong to the orthorhombic space group P222, with cell parameters of ca 55.88 x 174.30 x 70.14 mm^3. Almost complete data to 2.4 Å has been obtained, but we have not been able to solve the structure with molecular replacement yet.
TAB5 is a zinc containing protein. It has been tried to collect data with anomalous signals, but the results have not been good enough to solve the structure.

The structure of DNase from shrimp

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 maximum 2.4 Å at SNBL. The crystals tend to diffract anisotropically, the reflections tend to be elongated and it has been difficult to determine whether the crystals were of orthorhombic or monoclinic space group. Data collected recently suggested that the space group should be P21212 and with cell parameters of 44 x 47 x 184 $Å^3$. Complete data has been collected to 2.6 Å on the native protein, but data with anomalous signals is required to solve the structure. Soaking or co-crystallization with heavy atoms yielding anomalous signals has not been successful yet.

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 were difficult to index and could be P21, C2 or C222

Several crystals have been tested and three data sets has been collected, one on a potential WO_4^{2-} derivative, and two on a potential Yb derivative. The P21 cell is 81 x 164 x 81 mm^3 and β=108. The data (WO_4^{2-} derivative) that could be processed was 97% complete to 2.8 Å. Unfortunately no anomalous signal was found to phase the structure.

Crystallographic studies of lactate oxidase from Aerococcus viridans
The crystals of lactate oxidase are are fragile and tend to crack when soaked in cryo solution prior to data collection. As a result, the diffraction pattern tend to be bad and difficult to index if possible at all. Data has been tried collected at room temperature, but the crystals tend to die before the data is complete.
The crystals do, however, diffract to about 2.5 Å at SNBL, and belong to space group C2 with cell parameters of 135.4 x 118.7 x 107.4 mm^3, β=120.9.

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.

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.

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.

Poster and other presentations including SNBL data

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.

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.