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**: 01-02-643
Beamline: BM01A	**Date of experiment**: from: 18.07.03 to: 21.07.03 and 28.11.03 to: 02.12.03	**Date of report**: 24.08.04
Shifts: 9 + 12	**Local contact(s)**: Dr. Jon Are BEUKES	*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 40 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 01-02-643 was allocated 9 + 12 shifts in 2003. Three scientists (Ole A. Andersen, Ronny Helland and Ellen Wang) collected data on the following proteins:

1. ALP-TAB5 (2 +1 sets)
2. DNase (1 set)
3. Protein A ((Glycosylated protein from salmon)1 set)
4. Human UDG mutant (1 set)
5. Proteinase X (1 set)

Several other proteins, including lactate oxidase and isocitrate dehydrogenase from various bacterial sources, were tested, but the crystals were unfortunately not of diffraction quality.

Human UDG mutant
Good data was collected to 1.8Å. Space group was P212121, with unit cell 47.51 x 54.77 x 78.07 mm^3. 105 degree data was collected. Data was integrated in DENZO and scaled in SCALA (CCP4). The data was 93% complete, R-sym was ca 13%, I/σI was ca 4 and multiplicity was ca 2.3. The structure was solved by molecular replacement in CNS using human UDG (PDB 1EMH) as search model. Rigid body refinement, simulated annealing and alternate cycles of model building in O and conjugated gradient refinement brought the R-factor to 20.9% and R-free to 25.5%.
The paper including this protein is shortly to appear in Journal of Molecular Biology:
Elin Moe, Ingar Leiros, Ellen Kristin Riise, Magne Olufsen, Olav Lanes, Arne Smalåscand Nils Peder Willassen. ”Optimisation of the surface electrostatics as a strategy for cold adaptation of uracil-DNA N-glycosylase (UNG) from Atlantic cod (*Gadus morhua)*”.

Proteinase X
Good data was collected to 1.65 Å at SNBL. Space group was C2, with unit cell of 88 x 42 x 74 mm^3, β=110.2°. 180 degree data was collected, and integration was carried out in DENZO. Scalepack and Scala (CCP4i) was used for scaling. The data was 100% complete, R-sym was ca 11%, I/σI was ca 6 and multiplicity was ca 3.7. The structure was solved using MolRep of CCP4, using proteinaseK (PDB 1IC6) as search model. The final R-factors are 14.7% (R-work) and 18.0% (R-free).
The protein is currently being compared to ProteinaseK and the manuscript is in preparation.

DNase
Data was collected to 2.4 Å, but space group could not be determined and further work on this dataset was abandoned.

ProteinA
Data was collected to 2.9 Å, but indexing failed.

Alcaline phosphatase (ALP-TAB5).
Data was collected to ca 3Å. None of the datasets were of such quality that the structures could be solved.

Publications:
Data collected at SNBL and ESRF has resulted in the following publications in 2003/2004:

2003

Andersen OA, Stokka AJ, Flatmark T, Hough E. (2003) 2.0A resolution crystal structures of the ternary complexes of human phenylalanine hydroxylase catalytic domain with tetrahydrobiopterin and 3-(2-thienyl)-L-alanine or L-norleucine: substrate specificity and molecular motions related to substrate binding. *J Mol Biol*. **333**(4), 747-757.

Leiros, I., Moe, E., Lanes, O., Smalås, A.O., & Willassen, N.P. (2003). "*The crystal structure of uracil-DNA-glycosylase from Atlantic cod (Gadus morhua) reveals cold-adaptation features*". *Acta Cryst*. **D59**, 1357-1365.

Heikinheimo, P., Helland, R., Leiros, H.K., Leiros, I., Karlsen, S., Evjen, G., Ravelli, R., Schoehn, G., Ruigrok, R., Tollersrud, O.K., McSweeney, S. and Hough., E. (2003). "*The Structure of Bovine Lysosomal alpha-Mannosidase Suggests a Novel Mechanism for Low-pH Activation*." *J. Mol. Biol.* **327**(3), 631-644.

Heikinheimo, P., R. Helland, Leiros, H.K., Leiros, I., Karlsen, S., Evjen, G., Ravelli, R., Schoehn, G., Ruigrok, R., Tollersrud, O.K., McSweeney, S. and Hough., E. (2003). "*Structure of the Bovine Lysosomal a-mannosidase, the Enzyme Involved in the Lysosomal Storage Disease a-Mannosidosis*." *ESRF Highlights 2002*: 12-13.

Helland, R., Czapinska, H., Leiros, I., Olufsen, M., Otlewski, J. & Smalås, A.O. (2003) "*Structural Consequences of Accomodation of Four Non-cognate Amino Acid Residues in the S1 Pocket of Bovine Trypsin and Chymotrypsin*" *J. Mol. Biol.* 333, 845-861.

2004

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.

Elin Moe, Ingar Leiros, Ellen Kristin Riise, Magne Olufsen, Olav Lanes, Arne Smalåscand Nils Peder Willassen. "Optimisation of the surface electrostatics as a strategy for cold adaptation of uracil-DNA N-glycosylase (UNG) from Atlantic cod (*Gadus morhua)*". *Journal of Molecular Biology*, In press

Honorata Czapinska, Ronny Helland, and Arne O. Smalås, Jacek Otlewski "Chymotrypsin S1 pocket revisited. Crystal structures of five new bovine chymotrypsin complexes with P1 BPTI variants". *Journal of Molecular Biology*, In press

Thesis

Riise, E.K. "*Structure/function studies of mutants of Uracil-DNA glycosylase from Atlantic Cod*." Cand. scient. oppgave, Universitetet i Tromsø, oktober 2003.