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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:**01-02-726**
Beamline: BM01A	**Date of experiment**: from: 31.08.05 to: 03.09.05	**Date of report**: 07.10.05
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.

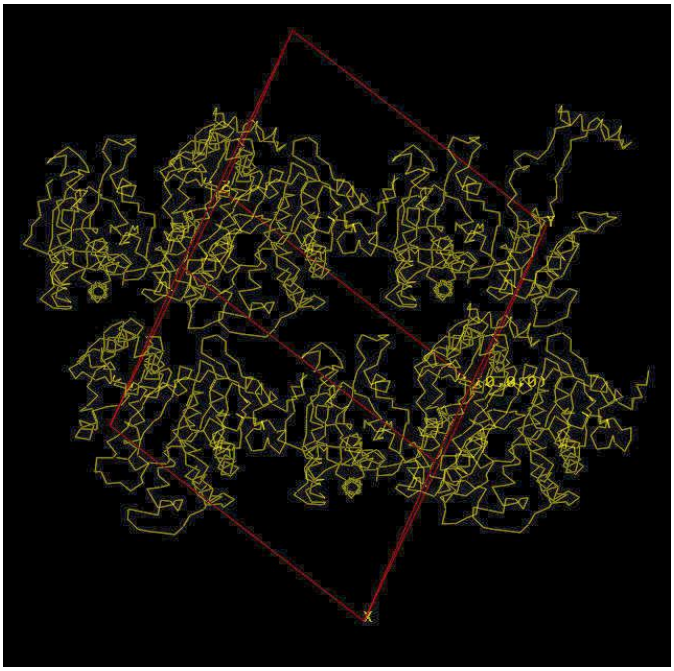
Data collection
Experiment 01-02-726 was allocated 9 shifts in September 2005. Crystals of five different proteins, both in native form and in complex, were tested for diffraction. Seven of the crystals were found to be suitable for data collection:

1. Isocitrate dehydrogenase from *Pyrococcus furiosus* (IDH1, 3 sets)
2. Isocitrate dehydrogenase from *Clostridium thermocellum* (IDH5, 2 sets)
3. Phenylalanine hydroxylase from *Colwellia psychrerythraea* (PAH, 2 sets)

Results
Structure of Isocitrate dehydrogenase from Pyrococcus furiosus
IDH1 can be crystallized from 0.4 M NH_4HPO_4 and 50 mM $MgSO_4$. Identical crystallization conditions appear to give two different crystal forms where one is monoclinic C2 and the other is trigonal or hexagonal. Data has previously been collected on this protein. Although the structure was solved, diffraction to 2.5 Å is not high enough for extensive comparative studies

The best crystal in this run diffracted to 2.2 Å. Data were collected in MAR 150 x 240 mode over 190 frames using oscillation of 1.0° and exposure of 35 sec. The best crystal belonged to space group C2 with cell parameters of 100.9 x 57.9 x 82.6 $Å^3$, b=115.5.
The second best crystal diffracted to ca 2.4 Å and appears to belong to a trigonal or hexagonal space group with an extremely long c-axis (cell parameters of 100.08 x 100.08 x 440.04$Å^3$). Data were collected over 900 frames with ocillation of 0.1° and exposure of 20 sec.

The structure of the first crystal form has been solved by molecular replacement (fig below)and refinement and model building is in progress.
The scaling statistics of the second crystal appears to be good, but the structures has not been solved yet, primarily because of problems with determining the right space group.



Structure of Isocitrate dehydrogenase from Clostridium thermocellum
IDH5 can be crystallized from 8% Peg3350, 10% PPG, 0.15M $(NH_4)_2HCit$ and the crystals are long thin hexagonal rods. Diffraction on this protein has been observed before, but only to ca 3 Å.

Data were collected on a native IDH5 crystal and on a crystal that had been soaked with substrate and cofactor. Both crystals diffracted to about 2.6 – 2.7 Å and belonged to a trigonal/hexagonal space group with approximate cell parameters of 130 x 130 x 60 $Å^3$. Data were collected in MAR 150 x 240 mode over 135 frames using oscillation of 0.75° and exposure of 45 sec in both sets. Both data sets were close to 100%

complete. Scaling statistics were acceptable for the native data (R-merge of ca 6%) but slightly worse for the complex (R-merge of ca 11%).
The structures have not been phased yet, probably because of problems in determining the right space group.

Structure of Phenylalanine hydroxylase from Colwellia psychrerythraea
Data were collected on two crystals soaked with cofactor. Both crystals belonged to the monoclinic space group P21 with approximate cell parameters of 40.4 x 86.7 x 88.1 $Å^3$, b=96.8. Data were collected in MAR 150 x 240 mode over 230 and 150 frames, crystals 1 and 2 respectively, using oscillation of 0.75° and exposure of 50 – 60 sec. Both crystals have been phased. The first crystal was identical to the native structure, but the second structure appears to have lost an important Fe-ion. Further refinement and model building is in progress on the second crystal.